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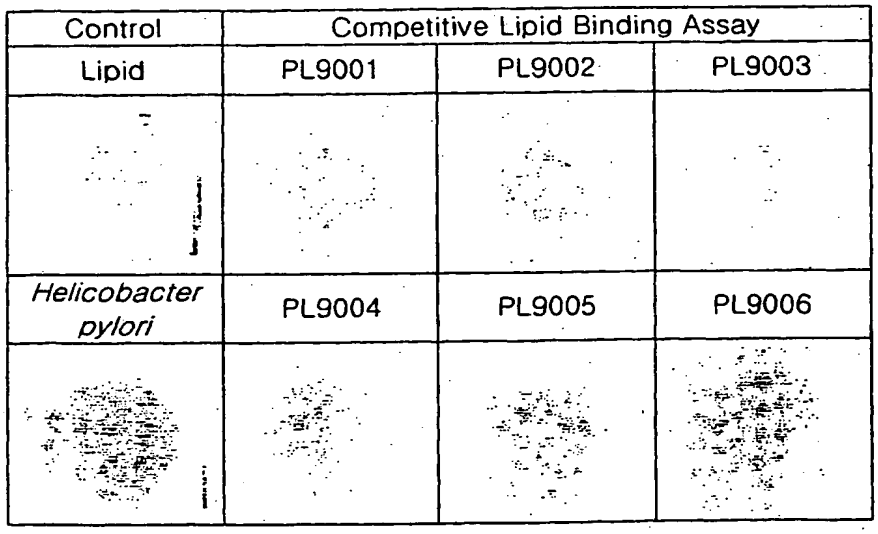
(54) Title: LACTIC ACID BACTERIA INHIBITING ADHESION OF HELICOBACTER PYLORI TO GASTRIC MUCOSA



(57) Abstract: The present invention relates to lactic acid bacteria capable of inhibiting activities of Helicobacter pylori, and more particularly, to lactic acid bacteria having an inhibitory activity on growth of Helicobacter pylori causing stomach ulcer and adhesion to the gastric mucosa, wherein the lactic acid bacteria are selected from the group consisting of coprophilus PL 9001(KCCM-10245), Enterococcus durans PL 9002 (KCCM-10246), Streptococcus faecalis PL 9003 (KCCM-10247), Lactobacillus coprophilus PL 9004 (KCCM-10248), Lactobacillus fermentum PL 9005 (KCCM-10250), and Lactobacillus fermentum PL 9006 (KCCM-10251). The lactic acid bacteria of the invention can be used as antiulcer drug, food additives, drugs for the prevention or treatment of Helicobacter pylori, drugs against bacteria that cause food poisoning, or drugs for the prevention or treatment of infectious bacteria.

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LACTIC ACID BACTERIA INHIBITING ADHESION OF *HELICOBACTER PYLORI* TO GASTRIC MUCOSA

BACKGROUND OF THE INVENTION

(a) Field of the Invention

The present invention relates to lactic acid bacteria capable of inhibiting activities of *Helicobacter pylori*, and more particularly, to lactic acid bacteria having an inhibitory activity on growth of *Helicobacter pylori* causing stomach ulcer and adhesion to the gastric mucosa.

(b) Description of the Related Art

Helicobacter pylori is a gram-negative bacterium. In 1983, Drs. Barry Marshall and Robin Warren of Australia discovered the bacteria in the stomach lining of patients with chronic gastritis and peptic ulcers. *Helicobacter* was initially called *Campylobacter pyloridis*, and was later named as *Helicobacter pylori* from its morphology in the body and the name of its habitat, the *pylorus.*

Helicobacter pylori infection is an important environmental factor in the pathogenesis of peptic ulcer disease, gastric carcinoma, and gastric lymphoma (Dubois, A. 1995. Spiral bacteria in the human stomach: the gastric *Helicobacters. Emerg. Infect. Dis.* 1: 79-85; Slomiany, B. L. and A. Slomiany. 1992. Mechanism of *Helicobacter pylori* pathogenesis: focus on mucus. *J. Clin. Gastroenterol.* 14 Suppl 1 S114-21). Once *Helicobacter*

pylori infects, it remains for several decades and is not eliminated naturally, and thus *Helicobacter pylori* is a major cause of chronic gastritis.

Helicobacter pylori infects through the intake of food, and it attaches to the gastric mucosa and the duodenal mucosa. A pathogenic factor of *Helicobacter pylori* is a urease that makes it for surviving in the highly acidic condition of a stomach, a flagellum for maintaining mobility, and the outer membrane protein having adherence to the gastric mucosa.

In its attachment to the human gastric epithelium, *Helicobacter pylori* binds with the same antigens identified as that of red blood cells (Alkout, A. M., C. C. Blackwell, D. M. Weir, I. R. Poxton, R. A. Elton, W. Luman, and K. Palmer. 1997. *Gastroenterol.* 112: 1179-1187; Boren, T., P. Flak, K. A. Roth, G. Larson, and S. Normark. 1993. *Science* 262: 1892-1895; Clyne, M. and B. Drumm. 1997. *Gastroenterol.* 113: 72-80). Antigens, like the Lewis antigen isolated in human blood type O, are expressed in the gastric mucosa. Therefore, gastritis breaks out mostly in blood-type O patients. (Heneghan, M. A., A. P. Moran, K. M. Feeley, E. L. Egan, J. Goulding J, C. E. Connolly, and C. F. McCarthy. 1998. *FEMS Immunol. Med. Microbiol.* 20: 257-266; Kobayashi, K., J. Sakamoto, Y. Yamamura, T. Kito, H. Inagaki, T. Watanabe, and H. Nakazato. 1991. *Nippon Geka Gakkai Zasshi* 92: 813-819; Kobayashi, K., J. Sakamoto, Y. Kito, Y. Yamamura, T. Koshikawa, M. Fugita, T. Watanabe, and H. Nakazato. 1993. *Am. J. Gastroenterol.* 88: 919-924).

For the treatment of *Helicobacter pylori* infection, antibiotics, inhibitor

of proton pumping, and antacid have been used. Because *Helicobacter* is difficult to culture with a large scale, a vaccine using the whole bacterium has been developed unsuccessfully. The method using antibiotics has disadvantage in that the *Helicobacter pylori* become resistant to the antibiotics and can be infected again. Method using antacid that suppresses secretion of gastric acid is not a basic solution. In addition, although a vaccine using urease has been developed, it is not effective. In the future, the development of a vaccine against *Helicobacter pylori* will be difficult, due to its complex culture conditions, which make it difficult to determine an active area for a vaccine.

SUMMARY OF THE INVENTION

The present invention is designed by the necessities of the prior art, and it is an object of the present invention to provide bacteria that are capable of suppressing stomach ulcers.

It is another object to provide bacteria that are capable of inhibiting the adhesion of *Helicobacter pylori* to the gastric mucosa.

It is still another object to provide bacteria that inhibit the activity of *Helicobacter pylori*.

It is still another object to provide bacteria that inhibit the growth of *Helicobacter pylori*.

It is still another object to provide bacteria that inhibit the growth of bacteria that cause food poisoning.

It is still another object to provide bacteria that inhibit the growth of anaerobic bacteria or bacteria that cause acne.

It is still another object to provide bacteria that improve immunity.

In order to achieve these objects, the present invention provides a bacterium for inhibiting the adherence of *Helicobacter pylori* to the gastric mucosa, wherein the bacterium is at least one selected from the group consisting of *Lactobacillus coprophilus, Enterococcus durans, Streptococcus faecalis*, and *Lactobacillus fermentum*.

The present invention provides a bacterium for inhibiting the growth of *Helicobacter pylori*, wherein the bacterium is at least one selected from the group consisting of *Lactobacillus coprophilus, Enterococcus durans, Streptococcus faecalis*, and *Lactobacillus fermentum*.

The present invention provides a composition for inhibiting the growth of bacteria, wherein the composition comprises at least one bacteria selected from the group consisting of *Lactobacillus coprophilus, Enterococcus durans, Streptococcus faecalis*, and *Lactobacillus fermentum*.

The present invention provides a cosmetic composition comprising at least one bacteria selected from the group consisting of *Lactobacillus coprophilus, Enterococcus durans, Streptococcus faecalis*, *Lactobacillus fermentum*, and a culture filtrate of bacteria thereof.

The present invention provides a food additive comprising at least one bacteria selected from the group consisting of *Lactobacillus coprophilus,*

Enterococcus durans, Streptococcus faecalis, *Lactobacillus fermentum*, and a culture filtrate of bacteria thereof.

The present invention provides a food prepared by fermenting at least one bacteria selected from the group consisting of *Lactobacillus coprophilus, Enterococcus durans, Streptococcus faecalis*, and *Lactobacillus fermentum*.

The present invention provides a immunological enhancement composition comprising at least one bacteria selected from the group consisting of *Lactobacillus coprophilus, Enterococcus durans, Streptococcus faecalis*, *Lactobacillus fermentum*, and a culture filtrate of bacteria thereof.

The present invention provides a composition for curing intestinal disorders comprising at least one bacteria selected from the group consisting of *Lactobacillus coprophilus, Enterococcus durans, Streptococcus faecalis*, *Lactobacillus fermentum*, and a culture filtrate of bacteria thereof.

BRIEF DESCRIPTION OF THE DLIVEINGS

FIG. 1 is a slide showing *Helicobacter pylori* attachment to glycolipids isolated from red blood cells, wherein the amount of adhered *Helicobacter pylori* is proportional to the amount of glycolipids (0 ug, 0.14 ug, 1.4 ug, 14 ug, 70 ug, 140 ug, 280 ug);

FIG. 2 is TLC plate that shows inhibition of *Helicobacter pylori* adhesion to glycolipids by PL bacteria;

FIG. 3 is a photograph showing inhibition of *Helicobacter pylori*

adhesion to the gastric mucosa by PL bacteria;

FIG. 4 is a photograph showing PL bacteria adhered to an MKN-45 cell;

FIG. 5 shows that materials produced from PL bacteria suppress the growth of *Helicobacter pylori*;

FIG. 6 is a graph showing the diameter of the growth-inhibited-zone for *Helicobacter pylori*;

FIG. 7 is a graph showing the growth-inhibited degree of *Helicobacter pylori* by a culture filtrate of PL bacteria;

FIG. 8 is a graph showing growth inhibition of bacteria that cause food poisoning by *Lactobacillus coprophilus* PL 9001;

FIG. 9 is a graph showing growth inhibition of bacteria that cause food poisoning by *Enterococcus durans* PL 9002;

FIG. 10 is a graph showing growth inhibition of bacteria that cause food poisoning by *Streptococcus faecalis* PL 9003;

FIG. 11 is a graph showing growth inhibition of bacteria that cause food poisoning by *Lactobacillus coprophilus* PL 9004;

FIG. 12 is a graph showing growth inhibition of bacteria that cause food poisoning by *Lactobacillus fermentum* PL 9005;

FIG. 13 is a graph showing growth inhibition of bacteria that cause food poisoning by *Lactobacillus fermentum* PL 9006;

FIG. 14 is a graph showing growth inhibition of bacteria that cause acne by PL bacteria;

FIG. 15 is a graph showing an immunological enhancement effect of PL bacteria; and

FIG. 16 is photograph showing the adhered PL bacteria to CaCo-2 cell lines.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

The present invention provides bacteria that inhibit the adhesion of *Helicobacter pylori* to the gastric mucosa. The bacteria are lactic acid bacteria and preferably *Lactobacillus sp.* or *Enterococcus sp.* More preferably, the bacteria are *Lactobacillus coprophilus*, *Enterococcus durans*, *Streptococcus faecalis,* and *Lactobacillus fermentum,* and most preferably, *Lactobacillus coprophilus* PL 9001, *Enterococcus durans* PL 9002, *Streptococcus faecalis* PL 9003, *Lactobacillus coprophilus* PL 9004, *Lactobacillus fermentum* PL 9005, and *Lactobacillus fermentum* PL 9006. The *Streptococcus faecalis* is identical to *Enterococcus faecalis*: its name has been changed to *Streptococcus faecalis* PL 9003.

The PL bacteria (*Lactobacillus coprophilus* PL 9001, *Enterococcus durans* PL 9002, *Streptococcus faecalis* PL 9003, *Lactobacillus coprophilus* PL 9004, *Lactobacillus fermentum* PL 9005, and *Lactobacillus fermentum* PL 9006) have been internationally deposited in the Korean Culture Center of Microorganisms and given accession numbers. The accession numbers

given by the international depositary authority are KCCM-10245 for *Lactobacillus coprophilus* PL 9001, KCCM-10246 for *Enterococcus durans* PL 9002, KCCM-10247 for *Streptococcus faecalis* PL 9003, KCCM-10248 for *Lactobacillus coprophilus* PL 9004, KCCM-10250 for *Lactobacillus fermentum* PL 9005, and KCCM-10251 for *Lactobacillus fermentum* PL 6.

The present invention provides an antiulcer drug. The antiulcer drug contains *Lactobacillus sp.* or *Enterococcus sp.*, and more preferably it contains the PL bacteria. The *Lactobacillus sp.* or *Enterococcus sp.* have inhibitory activities for *Helicobacter pylori* adhesion to the gastric mucosa as well as for *Helicobacter pylori* growth. These activities are confirmed with *Lactobacillus coprophilus* PL 9001 (KCCM-10245), *Enterococcus durans* PL 9002 (KCCM-10246), *Streptococcus faecalis* PL 9003 (KCCM-10247), *Lactobacillus coprophilus* PL 9004 (KCCM-10248). *Lactobacillus fermentum* PL 9005 (KCCM-10250), and *Lactobacillus fermentum* PL 9006 (KCCM-10251).

Also, the PL bacteria produce materials that inhibit the growth of *Helicobacter pylori* and adhesion thereof to the gastric mucosa, and thus, a culture filtrate of the PL bacteria can be used for the anti-Helicobacter materials. The PL bacteria have antibiotics-resistant activity, acid-resistant activity, and bile-resistant activity, and are stable *in vivo*, and the PL bacteria can be provided as live bacteria, dehydrated bacteria, and non-viable bacteria.

The antiulcer drug of the present invention can be administered via oral or parenteral routes. Preferably, the drug would be administered orally or by injection, but most preferably, the drug would be administered orally. The antiulcer can be prepared as a single compound drug or a complex drug, and the complex drug can contain more pharmaceutically acceptable material. It is reasonable that the blending ratio of the PL bacteria is controlled according to the type of drug.

The culture filtrate of PL bacteria is a solution without bacteria that is prepared by filtering or centrifuging culture fluid, and more preferably, the culture filtrate is a supernatant phase prepared by inoculating the PL bacteria in a MRS liquid broth, culturing at 37℃ for 16-48 hr, and centrifuging.

In addition, *Lactobacillus sp*. and *Enterococcus sp*. can be applied to inhibit the activity of other bacteria. More preferably, the PL bacteria (PL9001, PL9002, PL9003, PL9004, PL9005, and PL9006), fragmented cell walls of the PL bacteria, or the culture filtrate of the PL bacteria can be used to inhibit the growth of bacteria that cause food poisoning, anaerobic bacteria, bacteria that cause acne, and so on.

The anaerobic bacteria are general disease-causing germs. The anaerobic bacteria are preferably tetanus bacteria, gas gangrene bacteria, and *Clostridium sp*., and more preferably, *Clostridium perfringens*.

The bacteria that cause food poisoning are bacteria such as a listeriosis, dysentery, diarrhea, 0157, and a bacteria that mediates food

poisoning.

PL bacteria of the present invention have activity for immunological enhancement, they have adhesion capability to gastric and intestinal cells, and they can be used to improve health. The PL bacteria can be administered to human beings or to animals for therapeutic or prophylactic treatment of the stomach and particularly the pylorus, for inhibiting growth of harmful bacteria, and for curing intestinal disorders. The PL bacteria and their culture filtrate are capable of displacing pathogenic bacteria from intestinal or gastric cells to which they adhere.

The present invention provides a composition including the PL bacteria. The composition can be used for inhibiting biological activity of *Helicobacter pylori* and bacteria, for immunity improving, or as a medicine for intestinal disorders, and it can be applied to feed, feed additives, food, food additives, medicine, or cosmetic compositions.

The composition contains the cell walls prepared by grinding the live, non-viable , and dehydrated PL bacteria or a culture filtrate thereof as an effective agent, and it further contains excipients or a carrier. The composition containing the excipients or carrier includes 0.001 to 90 wt% of PL bacteria, but it is not limited to that. The PL bacteria of the present invention have undergone a toxicity test, which has confirmed that the PL bacteria are safe *in vivo*.

The feed or feed additives include 0.001 to 90 wt% of PL bacteria or

a culture filtrate thereof, but its amount is not limited to that. The feed or feed additives are prepared as a drink or a solid product.

The food or food additives are preferably yogurt, weaning diet, dairy goods, cheese, Kimchi, drinks, or other food, and they are prepared by fermenting with the PL bacteria of the present invention as a seed.

The medicine may be used by itself or it may be included with more than one pharmaceutical composition. The medicine can include more than one kind of pharmaceutical diluent selected from the group consisting of saline, buffered saline, dextrose, water, glycerol, and ethanol, but the diluent is not limited. Appropriate diluents are listed in the written text of Remington's Pharmaceutical Science (Mack Publishing Co, Easton PA).

The medicine may be applied differently according to the purpose of dosing and diseases. It should be understood that the amount of the active ingredient actually administered ought to be determined in light of various relevant factors including the condition to be treated, the severity of the patient's symptoms, co-administration with other drugs (e.g., chemotherapeutic agents), the age, sex, and body weight of the individual patient, food consumed, dosing time, the chosen route of administration, and the ratio of the composition.

A daily dose of the medicine is preferable from about 1 to 800 mg, and most preferably 40 to 400 mg. In general, 0.1 to 20 mg/kg, and preferably 0.2 to 10 mg/kg of the medicine can be administrated in a single

or in 1-3 divided doses per day, even though the exact dose and route of administration are adjusted to the type and severity of disease.

The medicine containing PL bacteria or a culture filtrate thereof can be administered via oral or parenteral routes. Parenteral dosing means the administration of a drug through a route other than oral, which includes rectal, intravenous, intraperitoneal and intramuscular, intra-arterial, transdermal, nasal, inhalation, ocular, and subcutaneous introduction.

Pharmaceutical formulations may be prepared in any form, such as an oral dosage form, an injectable solution, suppository or a topical preparation. The formulation is preferably prepared for oral and injectable administration (true solution, suspension, or emulsion), and most preferably in oral form such as tablet, capsule, soft capsule, aqueous medicine, pill, granules, and the like.

In preparing the formulation, the PL bacteria or a culture filtrate are filled in the soft capsule without any excipients, or formed as an appropriate formulation after mixing or diluting with a carrier. Examples of suitable carriers are starches, water, saline, Ringer's solution, dextrose, and any ingredients described in previous reports (e.g. Remington's Pharmaceutical Science, Mack Publishing Co., Easton PA).

The cosmetic composition contains 0.001 to 20 wt% of PL bacteria or a culture filtrate thereof, but the ratio is not limited to that. The cosmetic composition further contains a general material added to cosmetics such as

oil, water, detergent, moisturizer, alcohol, thickener, chelate compound, pigment, preservative, and perfume, in appropriate amounts. The cosmetic compound can be formulated as a toner, lotion, cream, essence, pack, powder, ointment, suspension, emulsion, or spray. The cosmetic composition may be used for prevention and treatment of bacteria, such as those causing acne, and it is preferably for external application.

The present invention provides a material for inhibiting the growth of harmful bacteria. The material for inhibiting the growth of harmful bacteria is able to suppress the growth of Helicobacter pylori, anaerobic bacteria, bacteria that cause acne, or bacteria that cause food poisoning, and it is prepared as a culture filtrate in a bacteria culture. Thus, the material for inhibiting the growth of harmful bacteria of *Lactobacillus* sp. or *Enterococcus* sp., can also be used for the treatment and prevention of bacteria.

As mentioned above, *Lactobacillus coprophilus* PL 9001, *Enterococcus durans* PL 9002, *Streptococcus faecalis* PL 9003, *Lactobacillus coprophilus* PL 9004. *Lactobacillus fermentum* PL 9005, and *Lactobacillus fermentum* PL 9006 of the present invention suppress the growth and adhesion of *Helicobacter pylori*. Also, PL bacteria, fragments of PL bacteria, or a culture filtrate of PL bacteria can be used for the treatment and prevention of bacterial infection. PL bacteria and a culture filtrate of PL bacteria inhibit the growth of bacteria that cause food poisoning, bacteria that cause acne, and anaerobic bacteria, as well as enhance immunity effects,

and thereby PL bacteria and a culture filtrate of PL bacteria can be used for treatment and prevention of bacterial manifestations.

The present invention will be explained in more detail with reference to the following Examples. However, the following Examples are to illustrate the present invention and the present invention is not limited to them.

EXAMPLE 1: Isolation of PL bacteria

MRS broth (Difco, bacto proteose peptone No.3 10 g, bacto beef extract 10 g, bacto yeast extract 5 g, bacto dextrose 20 g, polysorbate 80 g, ammonium citrate 2 g, sodium acetate 5 g, magnesium sulfate 0.1 g, manganese sulfate 0.05 g, and dipotassium phosphate 2 g /L) was mixed with 0.002% of bromophenol blue so that a MRS+BPB medium was prepared.

Kimchi samples were diluted with a 10 X pepton solution. 0.1 mL of the diluted solution was inoculated into the MRS+BPB medium and spread. The feces from an infant were picked by a cotton swab and inoculated into the MRS+BPB medium. After incubation for 3-4 days in an incubator (25℃), lactic acid bacteria were observed and isolated according to the colony form.

In order to distinguish a group of lactic acid bacteria, a MRS solid medium was added to BPB which displays a yellow color at pH 3.0 and a purple color at pH 4.6, and the lactic acid bacteria were classified according to coloration by the BPB. The coloration was determined by producing-

degrees of lactic acid, pH-resistance, and the length of life. *P. acidolactic* and *S. faecalis* are normal lactic acid bacterium, *L. mesenteroides* and *L. brevis* are abnormal lactic acid bacterium, and *L. plantarum* is a random lactic acid bacterium. *S. faecalis* forms a white colony where its medium changes to light yellow. *L. mesenteroides* ferments abnormal lactic acid and produces a low level of lactic acid, and the colony is deep blue as a whole and has a small size without a ring. *Lactobacillus sp*. has a light blue ring, being deep blue in the center, or a generally white color, and the size of the colony is large. *P. acidolactic* and *L. mesenteroides* express a deep-blue because of a short lifetime and low pH. For example, *L. mesenteroides* cannot grow below pH 4.8.

All bacteria of the present invention formed a white colony of over 0.3 mm (diameter) and were classified as *Lactobacillus sp*.

Bacteria were isolated from a single colony, according to Bergy's manual of systematic bacteriology, by exhibition of morphological and biochemical properties. After performed a gram stain and a catalase-reaction, the bacteria were analyzed in an API system (La Balme-les-Grottes, France). After the colony was picked with a cotton swab, it was floated with 2 ml of distilled water. The floating solution was added to an API 50 CHL medium and mixed. 200 ul of the mixture were inoculated into each of 50 tubes, and each mixture was covered with mineral oil and incubated at 37℃ for 48 hr.

The fermentation pattern of 49 kinds of saccharides were analyzed with API 50 CH and API 50 CHL, the resulting data were fed into the ATB identification computer system (bio Merieux France), and then the bacterium was identified. The results of the first bacterium among the isolated microorganisms are presented in Table 1.

(Table 1)

Strip No.1 tube/substrate	Strip No.2 tube/substrate	Strip No.3 tube/substrate	Strip No.4 tube/substrate	Strip No.5 tube/substrate
- Control	- Galactose	- D-Mannoside	- Melibiose	- D-Turanose
- Glycerol	+ D-Glucose	- D-Glucoside	+ Saccharose	- D-Lyxose
- Erythritol	+ D-Fructose	+ Glucosamine	- Trehalose	- D-Tagatose
- D-Arabinose	+ D-Mannose	+ Amygdaline	- Inuline	- D-Fucose
- L-Arabinose	- L-Sorbose	+ Arbutine	- Melezitose	- L-Fucose
- Ribose	- Rhamnose	+ Esculine	- D-Raffinose	- D-Arabitol
+ D-Xylose	- Dulcitol	+ Salicine	- Amidon	- L-Arabitol
- L-Xylose	- Inositol	+ Cellobiose	- Glycogene	+ Gluconate
- Adonitol	- Mannitol	+ Maltose	- Xylitol	- 2-Gluconate
- Xyloside	- Sorbitol	- Lactose	+ Gentiobiose	- 5-Gluconate

The first bacterium has a 99.9% similarity to *Lactobacillus coprophilus* and a 0.1% similarity to *Lacto. brevis*. 16S rRNA of the first bacterium was sequenced with a 16S rRNA gene kit (Perkin Elmer Applied Biosystem) and the sequence is shown as Sequence No. 1. The base sequence of Sequence No. 1 is identical to the 16S rRNA base sequence of *Lactobacillus coprophilus* as found from a BLAST search (http://www.ncbi.nlm.nih.gov/blast). Other names of *Lactobacillus coprophilus* are *Weissella confusa* and *Lactobacillus confusus*. The bacterium was deposited under Lactobacillus 9001 (KFCC-11240) in the Korean Federation of Culture Collections, and internationally deposited

under *Lactobacillus coprophilus* PL 9001(KCCM-10245) in the Korean Culture Center of Microorganisms.

The second bacterium was characterized with an API STREP Kit and the results are presented in Table 2.

(Table 2)

VP	+	ARA	+
HIP	-	MAN	+
ESC	+	SOR	-
PYRA	+	LAC	-
AGAL	-	TRE	-
β GUR	-	INU	-
β GAL	-	RAF	-
PAL	-	AMD	-
LAP	-	GLYG	-
ADH	+	β - HEM	-
RIB	+		

For the non-classified second bacterium, the 16S rRNA base sequence was analyzed. The 16S rRNA base sequence of the second bacterium is Sequence No. 2, and it is identical to that of *Enterococcus durans*. According to the sequencing result, the second bacterium was called *Enterococcus durans* PL 9002, and it was deposited under *Enterococcus durans* PL9002 (KFCC-11241) in the Korean Federation of Culture Collections, and internationally deposited under *Enterococcus durans* PL 9002 (KCCM-10246) in the Korean Culture Center of Microorganisms.

The results of the third bacterium characterization are presented in Table 3.

(Table 3)

VP	+	ARA	-

HIP	-	MAN	+
ESC	+	SOR	+
PYRA	+	LAC	+
AGAL	-	TRE	+
β GUR	-	INU	-
β GAL	-	RAF	-
PAL	-	AMD	+
LAP	+	GLYG	-
ADH	+	β - HEM	-
RIB	+		

The third bacterium was identical to *Streptococcus faecalis* (*Enterococcus faecalis*) by 99.1%. The bacterium was deposited under *Streptococcus faecalis* 9003 (KFCC-11242) in the Korea Federation of Culture Collections, and internationally deposited under *Streptococcus faecalis* PL 9003(KCCM-10247) in the Korea Culture Center of Microorganisms.

The 16S rRNA base sequence of the third bacterium was analyzed as Sequence No. 3, and it was found to be identical to that of *Streptococcus faecalis* (*Enterococcus faecalis*).

The results of the fourth bacterium characterization are presented in Table 4.

(Table 4)

Strip No.1 tube/substrate	Strip No.2 tube/substrate	Strip No.3 tube/substrate	Strip No.4 tube/substrate	Strip No.5 tube/substrate
- Control	- Galactose	- D-Mannoside	- Melibiose	- D-Turanose
- Glycerol	+ D-Glucose	- D-Glucoside	+ Saccharose	- D-Lyxose
- Erythritol	+ D-Fructose	+ Glucosamine	- Trehalose	- D-Tagatose
- D-Arabinose	+ D-Mannose	+ Amygdaline	- Inuline	- D-Fucose
+ L-Arabinose	- L-Sorbose	- Arbutine	- Melezitose	- L-Fucose
- Ribose	- Rhamnose	+ Esculine	- D-Raffinose	- D-Arabitol
+ D-Xylose	- Dulcitol	+ Salicine	- Amidon	- L-Arabitol
- L-Xylose	- Inositol	+ Cellobiose	- Glycogene	+ Gluconate
- Adonitol	- Mannitol	+ Maltose	- Xylitol	- 2-Gluconate
- Xyloside	- Sorbitol	- Lactose	+ Gentiobiose	- 5-Gluconate

The fourth bacterium was identical to *Lactobacillus coprophilus* by 98.2% and to *Lacto. brevis* by 1.3%. Also, the 16S rRNA base sequence of the bacterium was analyzed as Sequence No. 4, and it was found to be identical to that of *Lactobacillus coprophilus* as a result of a BLAST search (http://www.ncbi.nlm.nih.gov/blast). The bacterium was deposited under *Lactobacillus coprophilus* PL 9004 (KFCC-11243) in the Korea Federation of Culture Collections, and internationally deposited under *Lactobacillus coprophilus* PL 9-4 (KCCM-10248) in the Korean Culture Center of Microorganisms

The results of the fifth bacterium characterization are presented in Table 5.

(Table 5)

Strip No.1 tube/substrate	Strip No.2 tube/substrate	Strip No.3 tube/substrate	Strip No.4 tube/substrate	Strip No.5 tube/substrate
- Control	+ Galactose	- D-Mannoside	+ Melibiose	- D-Turanose
- Glycerol	+ D-Glucose	- D-Glucoside	+ Saccharose	- D-Lyxose
- Erythritol	+ D-Fructose	+ Glucosamine	- Trehalose	- D-Tagatose
- D-Arabinose	+ D-Mannose	- Amygdaline	- Inuline	- D-Fucose
- L-Arabinose	- L-Sorbose	- Arbutine	- Melezitose	- L-Fucose
+ Ribose	- Rhamnose	- Esculine	+ D-Raffinose	- D-Arabitol
- D-Xylose	- Dulcitol	- Salicine	- Amidon	- L-Arabitol
- L-Xylose	- Inositol	- Cellobiose	- Glycogene	+ Gluconate
- Adonitol	- Mannitol	+ Maltose	- Xylitol	- 2-Gluconate
- Xyloside	- Sorbitol	- Lactose	- Gentiobiose	- 5-Gluconate

The fifth bacterium was identical to *Lactobacillus fermentum* by 93.2% and to *Leuoconostoc lactis* by 6.7%. Also, the 16S rRNA base sequence of the bacterium was analyzed as Sequence No. 5, and it was found to be identical to that of *Lactobacillus fermentum* as a result of a

BLAST search (http://www.ncbi.nlm.nih.gov/blast). The bacterium was deposited under *Lactobacillus fermentum* PL 9005 (KCCM-10250) in the Korean Culture Center of Microorganisms.

The results of the sixth bacterium characterization are presented in Table 6.

(Table 6)

Strip No.1 tube/substrate	Strip No.2 tube/substrate	Strip No.3 tube/substrate	Strip No.4 tube/substrate	Strip No.5 tube/substrate
- Control	+ Galactose	+ D-Mannoside	+ Melibiose	- D-Turanose
- Glycerol	+ D-Glucose	- D-Glucoside	+ Saccharose	- D-Lyxose
- Erythritol	+ D-Fructose	- Glucosamine	+ Trehalose	- D-Tagatose
- D-Arabinose	+ D-Mannose	- Amygdaline	- Inuline	- D-Fucose
- L-Arabinose	- L-Sorbose	- Arbutine	- Melezitose	- L-Fucose
+ Ribose	- Rhamnose	- Esculine	+ D-Raffinose	- D-Arabitol
- D-Xylose	- Dulcitol	- Salicine	- Amidon	- L-Arabitol
- L-Xylose	- Inositol	- Cellobiose	- Glycogene	- Gluconate
- Adonitol	- Mannitol	+ Maltose	- Xylitol	- 2-Gluconate
- Xyloside	- Sorbitol	+ Lactose	+ Gentiobiose	- 5-Gluconate

The sixth bacterium was identical to *Lactobacillus fermentum* by 94.4% and to *Lactobacillus lactis* by 5.4%. Also, the 16S rRNA base sequence of the bacterium was analyzed as Sequence No. 6, and it was found to be identical to that of *Lactobacillus fermentum* as a result of a BLAST search (http://www.ncbi.nlm.nih.gov/blast). The bacterium was deposited under *Lactobacillus fermentum* PL 9006 (KCCM-10251) in the Korean Culture Center of Microorganisms

EXAMPLE 2. Test for inhibiting adherence of *Helicobacter pylori* to the gastric mucosa

(1) Preparation of bacteria

Helicobacter pylori (ATCC 43504) was cultured for 48 hr on a Brucella solid medium (Brucella broth, fungizone (2.5 g/ml amphotericin B) with Skirrow's supplement (0.16 mg/ml polymyxin B, 5 mg/ml vancomycin, 2.5 mg/ml trimethoprim)) and further supplemented with 10% horse serum, under a 5-10% CO_2 incubator. Cultured cells were collected by scraping, they were washed twice with a phosphate-buffered saline (PBS, pH 7.4), and then kept in a 10 mM Tris-Cl buffer at -20℃ until used.

The bacteria prepared by EXAMPLE 1 is referred to as PL bacteria. The PL bacteria (*Lactobacillus coprophilus* PL 9001(KCCM-10245), *Enterococcus durans* PL 9002(KCCM-10246), *Streptococcus faecalis* PL 9003(KCCM-10247), *Lactobacillus coprophilus* PL 9004(KCCM-10248). *Lactobacillus fermentum* PL 9005 (KCCM-10250), and *Lactobacillus fermentum* PL 9006 (KCCM-10251)) were grown for 24 hr at 37℃ in an MRS broth, collected by centrifugation, pellet washed 2-3 times with PBS (pH 7.4), and then kept in 10 mM Tris-cl at -20℃ until used.

(2) Extraction and purification of glycolipid

Glycolipid was isolated from human type O red blood cells (RBCs), as described in a report by Lee et. al. (Lee, Y., E. Shin, J. Lee, and J.H. Park. 1999. *J. Microb. Biotech.* 9: 794-797), with a slight modification. The human type O RBCs were dispersed in a minimum volume of water, repeated processed by freezing at –70℃ and melting, until they were broken down. After the lysate was fixed, the supernatant was removed. The

remains were collected and extracted as mentioned below, and then mixed with a chloroform-methanol mixture (2:1, v/v), and a lower phase (containing lipid) was collected. The lipid layer material was dried in a rotary vacuum evaporator. The dried solid was dissolved in chloroform containing 2% methanol and loaded into a column (bed volume = 20 ml) that was equilibrated with silicic acid (H_2SiO_3). The fractions were collected by sequentially eluting with chloroform, acetone-methanol (3:1, v/v), and methanol. The methanol fraction was then dried in a rotary vacuum evaporator, dissolved in a minimum volume of methanol, and stored in microcentrifuge tube at –70℃.

(3) Competitive lipid binding assay

A competitive lipid binding assay (Lee, Y., E. Shin, J. Lee, and J.H. Park. 1999. *J. Microb. Biotech.* 9: 794-797) was performed with a slight modification. The extracted glycolipid (14 ug/5 ul) was spotted on a thin layer chromatography plate (TLC, Merck, Kreselgel60, EM Separations, Gibbstown, NJ, U.S.A). The plate was soaked in a 10 mM Tris buffer (pH 7.6) containing 3% gelatin for 2 hr at 37℃ to prevent non-specific binding. The TLC plate was rinsed twice with the same buffer and a culture solution of PL bacteria (OD_{600}=1, CFU 2.4 X 10^8) was added. After 5 to 30 min, *H. pylori* (2.0 X 10^8 CFU) was added and the mixture was gently agitated for 2 hr at 37℃. In addition, except for the PL bacteria, the control plate was reacted with only *Helicobacter pylori* as described above, and agitated for 2

hr. The plate was rinsed 3 times with the same buffer for 10 min/time. The first antibody, rabbit antiserum raised against *H. pylori,* was added to the buffer (1: 600) and further incubated for 2 hr at room temperature with gentle shaking. After the plate was washed to remove the first antibody (1: 1000), IgG, the second antibody conjugated with alkaline phosphatase, was added to the plate and the plate was incubated for 1 hr at room temperature. A chromogenic reaction was observed by adding BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate disodium salt/nitro blue tetrazolium chloride). Therefore, the chromogenic level showed the adherence degree of *Helicobacter pylori* on glycolipid derived from red blood cells.

FIG. 1 is a TCL plate showing that with *Helicobacter pylori* bound to glycolipid extracted from red blood cells, the amounts of bound *H. pylori* increased linearly as the amount of spotted glycolipid (0 ug, 0.14 ug, 1.4 ug, 14 ug, 70 ug, 140 ug, 280 ug) increased.

FIG. 2 is TLC plate for observing whether or not *Lactobacillus* inhibits the adhesion of *Helicobacter pylori* on glycolipids. PL bacteria (*Lactobacillus coprophilus* PL 9001 (KCCM-10245), *Enterococcus durans* PL 9002(KCCM-10246), *Streptococcus faecalis* PL 9003 (KCCM-10247), *Lactobacillus coprophilus* PL 9004 (KCCM-10248), *Lactobacillus fermentum* PL 9005 (KCCM-10250), and *Lactobacillus fermentum* PL 9006 (KCCM-10251)) was reacted with *Helicobacter pylori*. As shown in FIG. 2, PL bacteria of the EXAMPLE inhibited the adherence of *Helicobacter*

pylori.

(4) Test for inhibiting adherence of *Helicobacter* pylori to the gastric mucosa

Bacteria having biological activity and non-viable bacteria prepared by boiling at 75℃ for 15 min were prepared. The adhesion assay was conducted with the surviving bacteria and the non-viable bacteria on MKN-45 (Human Gastric Adenocarcinoma).

MKN cells (2.8-3.0 X 10^5) were added to each plate(diameter 30 mm) and cultured in a CO_2 incubator for 5-6 days until confluency. After cells were confluent, cells were washed three times with PBS (phosphate-buffered saline), a new medium was added, and cultured further 30 min at 37℃. Helicobacter pylori (10^6 CFU) and PL bacteria (10^7 CFU) were added to these MKN cells and incubated for 1 hour. Unbound PL bacterial cells were removed by three times washing with PBS. Then cells were fixed in a fixing solution (100 ml 3.5% formaldehyde, 16 g Na_2HPO_4, 4 g NaH_2PO_4.H2O, D.W. to 1 liter) and kept for 2 hours or overnight at 4 ℃. After fixation, cells were washed three times with PBS-Tween 20 (0.05% v/v) and blocked with 1 ml PBS-BSA (1%, w/v) in each plate for 30 min incubation at 37℃. Cells were washed three times with PBS-Tween 20. Then first antibody specific to *Helicobacter pylori* raised in rabbits were added (1:600 dilution) and kept for 1 hour at 37℃. Extra unbound first antibody was washed off by washing three times with PBS-Tween20. The secondary antibody which is the FITC

conjugate with mouse monoclonal anti-rabbit IgG (Sigma-Aldrich, Inc) were added (1:100 dilution) and cells were incubated for 1 hour at 37℃. cells were washed well with PBS-Tween 20 at least 3 times. After drying, cells were observed under a microscope (NIKON FDX-35, EX 450-490 nm, DM 505, BA 520 nm) and pictures were taken.

FIG. 3 is a photograph showing the inhibition of adherence of *Helicobacter pylori* to gastric cells by PL bacteria, wherein the part showing the yellow color in FIG. 3 is *Helicobacter pylori* labeled with FITC conjugated antibody under a fluorescence microscope. FIG. 3a is the MKN-45 cell, FIG. 3b is the MKN-45 cell treated with *Helicobacter pylori,* FIG. 3c is the MKN-45 cells treated with the surviving PL9001 bacterium and *Helicobacter pylori*, FIG. 3d is the MKN-45 cells treated with the non-viable PL9001 bacteria and *Helicobacter pylori*, FIG. 3e is the MKN-45 cell treated with the surviving PL9003 bacteria and *Helicobacter pylori*, FIG. 3f is the MKN-45 cells treated with the non-viable PL9003 bacteria and *Helicobacter pylori*, FIG. 3g is the MKN-45 cells treated with the surviving PL9004 bacteria and *Helicobacter pylori*, FIG. 3h is the MKN-45 cells treated with the non-viable PL9004 bacteria and *Helicobacter pylori*, FIG. 3i is the MKN-45 cells treated with the surviving PL9005 bacteria and *Helicobacter pylori*, FIG. 3j is the MKN-45 cells treated with the non-viable PL9005 bacteria and *Helicobacter pylori*, FIG. 3k is the MKN-45 cells treated with the surviving PL9006 bacteria and

Helicobacter pylori, and FIG. 3I is the MKN-45 cells treated with the non-viable PL9006 bacterium and *Helicobacter pylori*. In florescence microscopy, MKN-45 cell shows green and *Helicobacter pylori* shows yellow. In a black and white photograph, *Helicobacter pylori* shows more brightly than MKN-45 cells.

It was confirmed that the surviving PL bacteria and non-viable PL bacteria inhibit the adhesion of *Helicobacter pylori* to gastric cells. Therefore, PL bacteria prevent the inhabitation of *Helicobacter pylori* in the stomach so that it can treat and protect from *Helicobacter pylori*-associated disease.

EXAMPLE 3. The adhesion assay conducted with PL bacteria on gastric cell lines

Bacteria having biology activity and non-viable bacteria prepared by boiling at 75℃ for 15 min were prepared. The adhesion assay was conducted with the surviving bacteria and the non-viable bacteria on MKN-45 (Human Gastric Adenocarcinoma).

MKN-45 cells were cultured in an RPMI-1640 medium (Gibco-BRL, New York, N.Y.) containing 2 g/L sodium bicarbonate, 10% heat-inactivated fetal bovine serum, and an antibiotic-antimycotic, pH 7.2. A cell monolayer was prepared in a 30 mm dish (Nunc Inc., Roskilde, Denmark) for Gram staining by inoculating 3 X 10^5 viable cells into a 2 ml culture medium. The medium was replaced every other day. Cells were used for binding at late

post-confluence after 6 days of culture.

After six days the confluent monolayer was washed twice with 2 ml of PBS (phosphate buffered saline) before the adhesion assay. 100 μL PL bacteria (1 X 10^7 cfu/ml) were added to 2 ml of a complete medium. This suspension was added to each dish and incubated at 37℃ in 5% CO_2-95% air. After incubation for 60 min, the monolayer was washed twice with sterile PBS (pH 7.4), and fixed with methanol. After being stained with Gram staining, cells were examined under a light microscope.

FIG. 4 is photograph showing the adhesion of the surviving PL bacteria and the non-viable PL bacteria to gastric cell, wherein "a" is the surviving PL9001 bacterium, "a`" is the non-viable PL9001 bacterium, "b" is the surviving PL9003 bacterium, "b`" is the non-viable PL9003 bacterium, "c" is the surviving PL9004 bacterium, "c`" is the non-viable PL9004 bacterium, "d" is the surviving PL9005 bacterium, "d`" is the non-viable PL9005 bacterium, "e" is the surviving PL9006 bacterium, and "e`" is the non-viable PL9006 bacterium.

Therefore, PL bacteria of the present invention have excellent adherent activity for gastric cells regardless of their biological activity.

EXAMPLE 4

PL bacteria were treated with 5 M $LiCl_2$ (Mark S. TURNER, Peter TIMMS, Louise M. HAFNER, and Philip M. GIFFARD (1997) Journal of Bacteriology, vol. 179, No. 10, p. 3310-3316) to remove the protein of the

cell walls, and the adhesion assay on gastric cells was tested. The number of adhered PL bacteria was measured with a light microscope, and the values are represented in Table 7.

The group treated with $LiCl_2$ shows a decrease of adherence of bacteria, therefore it is confirmed that protein of cell walls of PL bacteria are involved in adhesion.

(Table 7)

		The observed Bacteria No.	Ratio (%)
The untreated group with $LiCl_2$		219.5/fields	100
The treated group with $LiCl_2$	PL9001	10.96/fields	54.8
	PL9003	100.0/fields	50.0
	PL9004	110.0/fields	55.0
	PL9005	98.0/fields	49.0
	PL9006	100.0/fields	50.0

EXAMPLE 5. Test of inhibiting *Helicobacter pylori* growth.

(1) Test of inhibiting *Helicobacter pylori* growth using a culture filtrate of PL bacteria.

The PL bacteria (*Lactobacillus coprophilus* PL 9001(KCCM-10245), *Enterococcus durans* PL 9002(KCCM-10246), *Streptococcus faecalis* PL 9003(KCCM-10247), *Lactobacillus coprophilus* PL 9004(KCCM-10248). *Lactobacillus fermentum* PL 9005 (KCCM-10250), and *Lactobacillus fermentum* PL 9006 (KCCM-10251)) was cultured for 24 h at 37℃ in MRS broth, and the supernatant was prepared as culture filtrate by centrifugation.

An inhibition test of *Helicobacter pylori* growth using the culture filtrate of PL bacteria was performed.

Wells were formed on a Brucella solid medium using a sterilized Pasteur pipet. *Helicobacter pylori* spread on the medium and the culture filtrate of PL bacteria was added to the well. After incubating in a CO_2 incubator (5% to 10% CO_2) for 2 days, the diameter of the zone that *Helicobacter pylori* growth inhibited due to the inhibitory material of the culture filtrate was measured.

FIG. 5 is a photograph showing that the culture filtrate has growth-inhibitory activity against *Helicobacter pylori*, and FIG. 6 shows a diameter of the growth-inhibited-zone of *Helicobacter pylori* by the culture filtrate. Therefore, it was confirmed that PL bacteria prepared by the EXAMPLE 1 secreted material that inhibited *Helicobacter pylori* growth.

(2) Test of inhibiting *Helicobacter pylori* growth by lyophilized culture filtrate of PL bacteria.

4 ml of MRS culture filtrate of PL bacteria was mixed with 2 ml of distilled MQ and the mixture was lyophilized. The lyophilized culture filtrate was suspended on 6 ml of skim milk (10 mg/ml) and after 1 ml of the solution was inoculated with 10 ul of *Helicobacter pylori* (OD_{625}=1.0), it was incubated for 1hr at 37℃ in a CO_2 incubator. The incubated solution was centrifuged (5000 rpm, 10min) and the supernatant was diluted by half with distilled MQ. The quantity of urease of the diluted solution was measured by an indolphenol method which assay the amount of ammonia produced from urea by urease. Since the quantity of urease is proportional to the amount

of *Helicobacter pylori*, the quantity of ammonia is proportional to the number of *Helicobacter pylori* in the incubated solution. Through these methods, growth inhibition of *Helicobacter pylori* by culture filtrate of PL bacteria was analyzed.

FIG. 7 is a graph showing the growth-inhibition degree of *Helicobacter pylori* by culture filtrate of PL bacteria, and it shows that the lyophilized culture filtrate of PL bacteria maintains the growth inhibition activity for *Helicobacter pylori*.

EXAMPLE 6. Toxicity test

The oral toxic test for the PL bacteria of EXAMPLE 1 was based on the "Toxic Test Standard for Drugs (1999. 12. 22)," Notification No. 1999-61 of the Korean Food and Drug Administration.

Tests were conducted on Sprague-Dawley rats, which were 5 weeks old (female : 100 ~ 120g / male : 110 ~ 130g). The rats were reared in 260 × 420 × 180 mm (W x L x H) cages at 23 ± 2℃ and 50 ± 10 humidity.

Referring to established rule No. 10 of the Korean Food and Drug Administration, when the value of LD_{50} is up to 5,000 mg/kg in an oral test, the material is a low toxicity material in a body. Therefore, in the experiment, to calculate the value of LD_{50} of PL bacteria, the dosage was set to 5,000 mg/kg (20 ml/kg B.W.) 5,000 mg/kg was the maximum dosage given to the rats to determine the toxicity of the material.

(Table 8)

Bacteria	Sex	number	Dosage(mg/kg B.W)	Dosage(ml/kg B.W)
I(PL 9001)	Male	5	5,000	20
	Female	5		
II(PL 9002)	Male	5	5,000	20
	Female	5		
III(PL 9003)	Male	5	5,000	20
	Female	5		
IV(PL 9004)	Male	5	2,500	20
	Female	5		
V(PL 9004)	Male	5	5,000	20
	Female	5		
VI(control)	Male	5	0	20
	Female	5		

After 14 days, no deaths were observed. Thus the value of LD_{50} could not be estimated.

Table 9 shows the weight change of the test group, and there were no significant changes.

(Table 9)

Sex	Days	I	II	III	IV	V	VI
M	0	120.1± 3.10	120.2± 3.08	120.1± 3.08	120.4± 2.86	120.2± 3.25	120.1± 3.31
	3	142.6± 6.81	146.9± 4.15	140.5± 5.46	140.7± 8.85	145.4± 7.73	143.0± 3.68
	7	162.7± 7.96	161.0± 5.87	155.5± 2.92	156.6± 11.59	162.1± 5.64	159.8± 3.06
	14	212.3± 8.40	193.1± 18.03	194.9± 9.14	201.1± 22.72	195.5± 11.14	193.6± 16.24
F	0	114.0± 4.34	113.9± 4.11	113.9± 4.17	113.5± 4.41	113.5± 4.45	113.6± 4.50
	3	134.3± 6.81	132.6± 4.15	131.0± 5.46	132.5± 8.85	129.8± 7.73	129.2± 3.68
	7	155.4± 8.01	151.5± 5.69	149.5± 5.86	152.2± 7.55	148.6± 5.81	148.8± 6.57
	14	199.8± 8.16	192.7± 10.87	192.5± 10.0	193.8± 12.06	194.7± 7.55	192.6± 9.35

In addition, all the rats were examined to determine abnormalities,

but there was no symptoms visible to ordinary sight (Table 10).

(Table 10)

Organ		I	II	III	IV	V	VI
Brain	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Kidney-Left	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Kidney-Right	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Heart	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Lung	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Spleen	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Liver	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Stomach	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Intestine	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Pancreas	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Adrenal gland(left)	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Adrenal gland(right)	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Pituitary gland	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Ovary-L	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Ovary-R	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5
Other organs	No. of observations	5	5	5	5	5	5
	No gross findings	5	5	5	5	5	5

As shown above, PL bacteria of the EXAMPLE is safe without oral toxicity and side effects.

EXAMPLE 7. Growth inhibition test for bacteria that cause food poisoning

An MRS culture filtrate of each PL bacterium was mixed with a 2 X concentrated BHI medium. Each of *Salmonella typhimurium, Salmonella*

Enteriditis, *Escherichia coli O157:H7*, *Aeromonas hydrophila*, and *Listeria monocytogenes* cultured in BHI (Brain Heart Infusion: Brain Heart, Infusion form, 6.0 g, Peptic digest of animal tissue 6.0 g, sodium chloride 5.0 g, dextrose 3.0 g, pancreatic digest of gelatin 14.5 g, disodium phosphate 2.5 g) were inoculated with a 1% final concentration of the mixture and incubated at 37℃. After 5 hr and 12 hr, the number of surviving bacteria was measured. For measurement, *Listeria monocytogenes* (L.M) was incubated in a blood-agar plate, and *Salmonella typhimurium* (S.T), *Salmonella Enteriditis* (S.E), *E. coli O157:H7* (O157), and *Aeromonas hydrophila* (A.H) were incubated in a MacConkey medium, and the number of surviving bacteria was measured at $O.D_{600}$.

Table 11 shows an $O.D_{600}$ of bacteria that cause food poison by a PL 9001 culture filtrate.

(Table 11)

Condition	0 hour	5 hour	24 hour
L.M	0.055	0.487	0.335
L.M + culture filtrate (PL9001)	0.059	0.069	0.068
S.T	0.053	0.538	0.748
S.T+ culture filtrate (PL9001)	0.063	0.073	0.072
S.E	0.053	0.572	0.966
S.E+ culture filtrate (PL9001)	0.059	0.073	0.07
O157	0.051	0.408	0.468
0157+ culture filtrate (PL9001)	0.06	0.074	0.065
A.H	0.077	0.418	0.432
A.H+ culture filtrate (PL9001)	0.069	0.093	0.083

FIG. 8 is a graph showing growth inhibition of bacteria that cause food poisoning by *Lactobacillus coprophilus* PL 9001, and it shows the

results of Table 11 (absorbance of $O.D_{600}$ at 0 hr was calculated to 100). As shown above in Table 11 and in FIG. 8, *Lactobacillus coprophilus* PL 9001 produced materials that inhibit the growth of bacteria that cause food poisoning.

Also, *Enterococcus durans* PL 9002 inhibited the growth of bacteria that cause food poisoning. Inhibition results are shown in Table 12 and FIG. 9.

(Table 12)

Condition	0 hour	5 hour	24 hour
L.M	0.055	0.487	0.335
L.M+ culture filtrate (PL 9002)	0.059	0.071	0.17
S.T	0.053	0.538	0.748
S.T+ culture filtrate (PL 9002)	0.064	0.073	0.196
S.E	0.053	0.572	0.966
S.E+ culture filtrate (PL 9002)	0.061	0.074	0.167
O157	0.051	0.408	0.468
0157+ culture filtrate (PL 9002)	0.59	0.073	0.17
A.H	0.077	0.418	0.432
A.H+ culture filtrate (PL 9002)	0.065	0.086	0.235

The inhibition effect of *Streptococcus faecalis* PL 9003 for growth of bacteria that cause food poisoning was measured. Table 13 shows $O.D_{600}$ of bacteria that cause food poisoning in a culture solution containing *Streptococcus faecalis* PL 9003, and the data of Table 13 are shown in the graph of FIG 10, showing that *Streptococcus faecalis* PL 9003 produced materials that inhibit the growth of bacteria that cause food poisoning.

(Table 13)

Condition	0 hour	5 hour	24 hour

L.M	0.055	0.487	0.335
L.M+ culture filtrate (PL 9003)	0.058	0.007	0.075
S.T	0.053	0.538	0.748
S.T+ culture filtrate (PL 9003)	0.063	0.074	0.084
S.E	0.053	0.572	0.966
S.E+ culture filtrate (PL 9003)	0.062	0.074	0.082
O157	0.051	0.408	0.468
0157+ culture filtrate (PL 9003)	0.058	0.07	0.071
A.H	0.077	0.418	0.432
A.H+ culture filtrate (PL 9003)	0.066	0.085	0.094

The inhibition effect of *Lactobacillus coprophilus* PL 9004 for the growth of bacteria that cause food poisoning was measured. Table 14 shows $O.D_{600}$ of bacteria that cause food poisoning in a culture solution containing *Lactobacillus coprophilus* PL 9004, and the data of Table 14 are shown in the graph of FIG. 11 of data of Table 14, showing that *Lactobacillus coprophilus* PL 9004 produced materials that greatly inhibit the growth of bacteria that cause food poisoning, but the inhibition effect was decreased.

(Table 14)

Condition	0 hour	5 hour	24 hour
L.M	0.055	0.487	0.335
L.M+ culture filtrate (PL 9004)	0.06	0.075	0.287
S.T	0.053	0.538	0.748
S.T+ culture filtrate (PL 9004)	0.063	0.071	0.266
S.E	0.053	0.572	0.966
S.E+ culture filtrate (PL 9004)	0.061	0.073	0.227
O157	0.051	0.408	0.468
0157+ culture filtrate (PL 9004)	0.062	0.073	0.267
A.H	0.077	0.418	0.432
A.H+ culture filtrate (PL 9004)	0.065	0.087	0.251

The inhibition effect of *Lactobacillus fermentum* PL 9005 for the growth of bacteria that cause food poisoning was measured. Table 15

below shows $O.D_{600}$ of bacteria that cause food poisoning in a culture solution containing *Lactobacillus fermentum* PL 9005, and FIG 12 is a graph showing the growth inhibition for Listeria. *Lactobacillus fermentum* PL 9005 produced materials that greatly inhibit the growth of bacteria that cause food poisoning.

(Table 15)

Condition	0 hr	5 hr	24 hr
L.M	0.055	0.487	0.335
L.M+ culture filtrate (PL 9005)	0.06	0.068	0.413
S.T	0.053	0.538	0.748
S.T+ culture filtrate (PL 9005)	0.065	0.074	0.434
S.E	0.053	0.572	0.966
S.E+ culture filtrate (PL 9005)	0.062	0.075	0.448
O157	0.051	0.408	0.468
O157+ culture filtrate (PL 9005)	0.06	0.077	0.389
A.H	0.077	0.418	0.432
A.H+ culture filtrate (PL 9005)	0.067	0.087	0.245

The inhibition effect of *Lactobacillus fermentum* PL 9006 for the growth of bacteria that cause food poisoning was measured. Table 16 shows $O.D_{600}$ of bacteria that cause food poisoning in a culture solution containing *Lactobacillus fermentum* PL 9006, and FIG 13 is a graph showing the growth inhibition for Listeria. *Lactobacillus fermentum* PL 9006 produced materials that greatly inhibit the growth of bacteria that cause food poisoning.

(Table 16)

Condition	0 hr	5 hr	24 hr
L.M	0.055	0.487	0.335
L.M+ culture filtrate (PL 9006)	0.058	0.068	0.283
S.T	0.053	0.538	0.748
S.T+ culture filtrate (PL 9006)	0.061	0.073	0.303
S.E	0.053	0.572	0.966

S.E+ culture filtrate (PL 9006)	0.065	0.069	0.428
O157	0.051	0.408	0.468
O157+ culture filtrate (PL 9006)	0.063	0.077	0.435
A.H	0.077	0.418	0.432
A.H+ culture filtrate (PL 9006)	0.067	0.087	0.112

As mentioned above, PL bacteria (*Lactobacillus coprophilus* PL 9001, *Enterococcus durans* PL 9002, *Streptococcus faecalis* PL 9003, *Lactobacillus coprophilus* PL 9004, *Lactobacillus fermentum* PL 9005 and *Lactobacillus fermentum* PL 9006) of the present invention produced materials that inhibit the growth of bacteria that cause food poisoning. Also, the decreased inhibition effect is caused by depleting material derived from culture filtrate, and then it can maintain the growth inhibition effect for bacteria that cause food poisoning by using PL bacteria.

EXAMPLE 8. Growth inhibition effect for bacteria that cause acne

Propionibacterium acne is a normal bacteria that exists on skin and causes acne. *Propionibacterium acne* (KCTC 3314) was inoculated into 5 ml of actinomyces broth, and after covering by parapin-oil, *Propionibacterium acne* was anaerobically incubated for 2 days (BR Vowels, S Yang, JJ Leyden. 1995. Induction of proinflammatory cytokines by a soluble factor of *Profionibacterium acnes*: Implications for chronic inflammatory acne. Infection and Immunity 63: 3158-3165). 45 ml of PL culture filtrate bred on a MRS liquid medium was mixed with 5 ml of *Profionibacterium acnes* culture solution. 50 ul of the mixture were inoculated into 5 ml of a fresh medium, an actinomyces broth, and cultured anaerobically for 2 days without shaking. For measurement of survival *Profionibacterium acnes,* the culture solution

was diluted (When OD_{600}=2.4, 9.6 X 10^8 CFU/ml) with a diluent containing 0.05% L-cysteine for anaerobic bacteria, and 100 ul of the diluted solution was inoculated onto an actinomyces agar plate. After culture in an anaerobic jar at 37℃ for 6-7 days, the colony on the plate was measured as the number of survival *Profionibacterium acnes.*

FIG. 14 is a graph showing the growth inhibition activity for bacteria that cause acne by PL bacteria, and *Lactobacillus coprophilus* PL 9001 did not inhibit nearly the growth of *Profionibacterium acnes*; *Enterococcus durans* PL 9002 and *Streptococcus faecalis* PL 9003 completely inhibited the growth of *Profionibacterium acnes*; and *Lactobacillus coprophilus* PL 9004, *Lactobacillus fermentum* PL 9005 and *Lactobacillus fermentum* PL 9006 inhibited the growth of *Profionibacterium acnes.*

EXAMPLE 9. Growth inhibition test for anaerobic bacteria.

Clostrodium perfringens was inoculated into BHI liquid broth containing Hemin (0.01 g/L) and L-cysteine (0.5 g/L) and it was anaerobically cultured at 37℃ for 24 hours (Balows A Hausler WJ Herrmann KL Isenberg HD Shadomy HJ. Chapter 50. Clostridium. p505-521. Manual of Clinical Microbiology, 5th ed. ASM Washington D.C. U.S.A.). A culture filtrate of PL bacteria was mixed with the same volume of 2X concentrated BHI broth, and a 1% final concentration of *Clostrodium perfringens* was inoculated into the mixture. After covering the mixture with parapin-oil, it was anaerobically cultured at 37℃. After 24 hours, the culture solution was diluted and

inoculated onto a blood agar plate. The plate was incubated for 37 days and 48 days, in an anaerobic incubator. The survival rate of *Clostrodium perfringens* was measured and is presented by log.

(Table 17)

	Number of survival bacteria (log cfu)	
	0 hrs	24 hrs
Clostrodium perfringens	6.60	8.30
Lactobacillus coprophilus PL 9001	6.60	7.60
Enterococcus durans PL 9002	6.78	7.66
Streptococcus faecalis PL 9003	6.60	7.48
Lactobacillus coprophilus PL 9004	6.90	8.00
Lactobacillus fermentum PL 9005	6.60	7.78
Lactobacillus fermentum PL 9006	6.60	7.41

It was confirmed that all the PL bacteria produced materials that inhibit growth of anaerobic bacteria.

EXAMPLE 10. Improving effect of immunity

The immune-improving effect of PL bacteria was tested. When PL bacteria was added to a macrophage, the amount of the producing cytokine (TNF-α , IL-6) was quantified.

50 ul of a coating buffer containing purified rabbit anti-TNF- α or rabbit anti-IL-6 was coated within a microplate well and the microplate was maintained at –4℃ for 12 hr. The microplate well was washed three times with a PBST (tween80+phosphate buffer) solution and reacted with 3% BSA-PBST, for 30 min. The microplate well was washed four times with PBST and reacted with 50 ul of TNF- α -standard/IL-6 standard (recombinant mouse TNF- α /IL-6, SEROTEC, UK) or macrophage supernatant cultured

in a medium containing PL bacteria, at 37℃ for 1 hr. The microplate well was washed four times with PBST, once with distilled water, and it was reacted with 50 ul of a mixture prepared by adding 3% BSA-PBST to biotinylated rat anti-mouse TNF-α or biotinylated rat anti-mouse TNF- α /IL-6 (biotinylated rat anti-mouse TNF-α /IL-6, 1 ug/ml), at room temperature for 1 hr. The microplate well was washed six times with PBST, once with distilled water, and reacted with streptavidin peroxidase for one hour. The microplate well was washed eight times with PBST, twice with distilled water, and reacted with TMB substrate (25 ml citric-phosphate buffer+400 ul TMB stock+100 ul of 1% H_2O_2) at room temperature until color showed. The reaction was stopped to add 6 N of H_2SO_4, and for measurement of immune reactivity, adsorption at OD_{450} was observed.

FIG. 15 and Table 18 show an immune-improving effect of PL bacteria, and a PL bacteria increase of the production of TNF-α and IL-6.

(Table 18)

		Adsorption at $O.D_{450}$		Increase ratio %
		Control	Test	
PL9001	TNF- α	15	136	9.06
	IL-6	0.5	21.5	43
PL9003	TNF- α	15	58	3.87
	IL-6	0.5	16.5	33
PL9004	TNF- α	15	88	5.87
	IL-6	0.5	18	36
PL9005	TNF- α	15	38	2.3
	IL-6	0.5	21.5	43
PL9006	TNF- α	15	59	3.93
	IL-6	0.5	21.4	42.8

Therefore, PL bacteria of the present invention can be applied to

promote immunity, and more particularly, it can be used for health food and as a treatment drug that promotes the health of aged persons and children.

EXAMPLE 11. Test for acid-resistance and bile-resistance

An MRS medium containing cysteine was optimized according to pH (7, 4.5 or 4.0) with 4 N HCl and 0.1 N NaOH, and was sterilized. To observe an effect of bile, oxgall powder was added to the medium in 0%, 0.25%, 0.50% concentrations and it was sterilized. Each PL bacteria was inoculated into the medium with a total of 1% concentration, and optical density was determined at 620 nm at 24-hours intervals. Table 19 shows the acid-resistance and bile-resistance. It was shown that PL 9003 was weak in acid, but PL 9001, PL 9002, PL 9004, PL 9005, and PL 9006 had strong acid-resistant activity, and all the PL bacteria had strong bile-resistant activity. Therefore, all PL bacteria are safe in the stomach and intestine (Conway PL Gorback SL goldin BR. 1987. Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. J. Dairy Sci. 70: 1-12. : Ibrahim SA Bezkorovainy A. 1993. Survival of bifidobacteria in the presence of bile salt. J. Sci. Food Agric. 62: 351-354).

(Table 19)

	Time	PL 9001	PL 9002	PL 9003	PL 9004	PL 9005	PL 9006
pH 7	24 hr	1.40	1.20	1.00	1.40	1.50	1.50
	48 hr	1.50	1.50	1.20	1.50	1.50	1.50
pH 5.0	24 hr	1.00	0.70	0.31	0.90	1.50	1.50
	48 hr	1.50	0.80	0.45	1.10	1.50	1.50
pH 4.5	24 hr	0.18	0.21	0.06	0.18	1.50	1.50
	48 hr	0.70	0.28	0.09	0.15	1.50	1.50
Oxgall 0.25%	24 hr	1.40	1.20	1.00	1.20	1.10	1.20
	48 hr	1.50	1.50	1.40	1.50	1.40	1.20

Oxgall 0.50%	24 hr	1.10	1.20	1.10	0.85	0.75	0.70
	48 hr	1.40	1.50	1.40	1.00	0.76	0.70

EXAMPLE 12. Antibiotic-resistance

Antibiotic-resistance for PL bacteria prepared by the EXAMPLE was observed. PL bacteria were inoculated into an MRS solid medium and a filter (diameter 6 mm) containing the antibiotic of Table 20 was put on the medium. After incubation for 24–48 hr, the diameter of the growth inhibited-zone formed by antibiotics was determined. A decreased diameter of the growth inhibited-zone means that PL bacteria have a resistance to the antibiotics. The results are presented in Table 20.

(Table 20)

Antibiotics	PL9001	PL9002	PL9003	PL9004	PL9005	PL9006
Penicillin (10 IU/E/UI)	20 mm	20 mm	24 mm	23 mm	30 mm	30 mm
Ampicillin (10 ug)	30 mm	24 mm	26 mm	30 mm	30 mm	32 mm
Cephalothin (30 ug)	30 mm	20 mm	20 mm	24 mm	30 mm	30 mm
Gentamycin (10 ug)	13 mm	-	-	12 mm	15 mm	16 mm
Vancomycin (30 ug)	-	20 mm	16 mm	-	-	-
Erythromycin (15 ug)	21 mm	-	-	22 mm	26 mm	29 mm
Tetracycline (30 ug)	26 mm	30 mm	10 mm	24 mm	27 mm	30 mm

As mentioned above, PL bacteria have high antibiotic resistance.

EXAMPLE 13. The adhesion assay conducted with PL bacteria on intestinal cell lines

Caco-2 cells (intestinal cell line) were cultured in an RPMI-1640 medium (Gibco-BRL, New York, N.Y.) containing 2 g/L sodium bicarbonate, 10% heat-inactivated fetal bovine serum, and antibiotic-antimycotic, pH 7.2. A cell monolayer was prepared in a 30 mm dish (Nunc Inc., Roskilde,

Denmark) for Gram staining by inoculating 3 X 10^5 viable cells into a 2 ml culture medium. The medium was replaced every other day. Cells were used for binding at late post-confluence after 6 days of culture.

After six days the confluent monolayer was washed twice with 2 ml PBS (phosphate buffered saline) before the adhesion assay. 100 μ L PL bacteria (1 X 10^7 cfu/ml) were added to 2 ml of a complete medium. This suspension was added to each dish and incubated at 37℃ in 5% CO_2-95% air. After incubation for 60 min, the monolayer was washed twice with sterile PBS (pH 7.4), and fixed with methanol. After being stained with Gram staining, cells were examined under a light microscope.

The number of observed bacteria within 20 fields was measured, and when their number was over 400 it was determined that adhesion occurred (C.N. JACOBSEN, V. ROSENFELDT NIELSEN, A.E. HAYFORD, P.L. MØLLER, K.F. MICHAELSEN, A. PAERREGAARD, B. SANDSTRÖM, M. TVEDE, and M. JAKOBSEN(1999) *Applied and Environmental Microbiology,* p.4949-4956 ; R. Bibiloni, P.F. Pérez, and G.L. DeAntoni (1999) *Anaerobe* 5, 483-485).

Table 21 shows a number of adhered PL bacteria. PL bacteria of the present invention are shown to have an adherence activity on intestinal cells.

(Table 21)

Name of the added PL bacteria	Attached bacteria No. on Caco-2
Bifidobacterium infantis	>800/20 fields

Bifidobacterium longum	>800/20 fields
PL9001	>800/20 fields
PL9003	>800/20 fields
PL9004	>800/20 fields
PL9005	>800/20 fields
PL9006	>800/20 fields

FIG. 16 is photograph showing the adhered PL bacteria on CaCo-2 cell lines. The adherence of PL9001 (b), PL9003 (c), PL9004 (d), PL9005 (e) and PL9006 (f) on intestinal cell lines was observed and compared to normal intestinal cell lines (a).

Bifidobacterium infantis and *Bifidobacterium longum* are the known adherent bacteria on intestinal cell lines, and compared to them, PL bacteria of the present invention are excellent adherent bacteria on intestinal cells and can therefore be applied to clean intestines.

EXAMPLE 16. Preparation of cosmetics

Preparation of toner

0.02 wt% powder prepared by drying PL 9001, 5.0 wt% glycerin, 3.0 wt% 1,3-butylene glycol, 1.0 wt% PEG1500, 0.1 wt% allanton, 0.3 wt% DL-pantenol, 0.02 wt% EDTA-2NA, 0.04 wt% benzopenon-9, 5.0 wt% sodium hyaluronate, 10.0 wt% ethanol, 16 wt% octyldodecene, 20 wt% polysorbate, antiseptic, pigment, and distilled water were mixed and toner was prepared.

Preparation of lotion

0.01 wt% of powder prepared by drying PL 9001, 5.0 wt% glycerin, 3.0 wt% 1,3-butyleneglycol, 5.0 wt% sodium hyaluronate, 10.0 wt% ethanol, 60wt% polysorbate, 1.5 wt% glycerylstearate 1.5 wt% stearyl alcohol, 1.5

wt% lanolin, 0.5 wt% sorbitan stearate, 1.0 wt% vegetable oils, 5.0 wt% mineral oil, 5.0 wt% squalene, 2.0wt% trioctanoin, 0.8wt% dimethicone, 0.5 wt% tocopherol acetate, 0.12 wt% carboxyvinylpolymer, 0.12 wt% triethanolamine, antiseptic, pigment, perfume, and distilled water were mixed and lotion was prepared.

Applicant's or agent's file reference	International application No.
OPP010966KR	

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 8, line 1.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution

KOREA CULTURE CENTER OF MICROORGANISMS

Address of depositary institution *(including postal code and country)*

361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu
SEOUL, 120-091, Republic of Korea

Date of deposit	Accession Number
December 2, 2000	KCCM-10245

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

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BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To. Yeon-hee Lee
Department of Biology and Culture Collection of Antibiotic Resistant Microbes, College of Natural Science, Seoul Woman's University, Seoul 139-774, Korea

RECEIPT IN THE CASE OF AN ORIGINAL issued pursuant to Rule 7.1 by the INTERNATIONAL DEPOSITARY AUTHORITY identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR : *Lactobacillus coprophilus* PL 9-1	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY : KCCM - 10245

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

☐ a scientific description

☐ a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

the microorganism identified under I above was received by this international Depositary Authority on Dec. 2. 2000. and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on Jan. 31. 2001.

IV. INTERNATIONAL DEPOSITARY AUTHORITY

Name : Korean Culture Center of Microorganisms Address : 361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu SEOUL 120-091 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s) : Date: Jan. 31. 2001

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BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSE OF PATENT PROCEDURE

ATTESTATION CONCERNING THE LATER INDICATION OR AN
AMENDMENT OF THE SCIENTIFIC DESCRIPTION AND/OR
PROPOSED TAXONOMIC DESIGNATION

pursuant to Rule 8.2

TO Yeonhee lee
Department of Biology,
Seoul woman's University,
Seoul 139-774,
Korea

The enclosed communication has been received by this International Depositary Authority on Jun. 21. 2001.

INDUSTRIAL DEPOSITARY AUTHORITY

Name : Korean Culture Center of Microorganisms
Address : 361-221, Yurim B/D
Hongje-1-dong,
Seodaemun-gu,
SEOUL 120-091
Republic of Korea

Signature(s) of person(s) having the power to represent the International Depositary Authority or authorized official(s) :

Date: Jun. 25. 2001.

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BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSE OF PATENT PROCEDURE

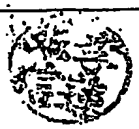
COMMUNICATION OF THE LATER INDICATION OR AN
AMENDMENT OF THE SCIENTIFIC DESCRIPTION
AND/OR PROPOSED TAXONOMIC DESIGNATION
pursuant to Rule 8.1

TO. KCCM
361-221 Yurim B/D
Hongje-1-dong Seodaemun-gu
Seoul. 120-091
Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM
Accession number given by the INTERNATIONAL DEOSITARY AUTHORITY: KCCM 10245
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION ☐[1]
☐[2] Scientific description: ☐[2] Last preceding scientific description (if any): ☐[2] Proposed taxonomic designation: Lactobacillus PL 9001 ☐[2] Last preceding proposed taxonomic designation (if any): Lactobacillus CCARM 9-1

[1]. Mark with a cross if additional information is given on an attached sheet.

[2]. Mark with a cross the applicable box or boxes.

III. REQUEST FOR ATTESTATION	
The undersigned 111 ☐[3] requests ☐[3] does not request the attestation referred to in Rule 8.2	
IV. DEPOSITOR	
Name: Yeonhee lee Address: Department of Biology, Seoul women's University, Seoul 139-774, Korea	Signature[4] :  Date: June. 21. 2001

[3]. Mark with a cross the application box.

[4]. Where the signature is required on behalf of a legal entity, the typewritten name(s) of the natural person(s) signing on the legal entity should accompany the signature(s).

Applicant's or agent's file reference	International application No.
OPP010966KR	

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

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Address of depositary institution *(including postal code and country)*

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SEOUL, 120-091, Republic of Korea

Date of deposit	Accession Number
December 2, 2000	KCCM-10246

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

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BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To. Yeon-hee Lee
Department of Biology and Culture Collection of Antibiotic Resistant Microbes, College of Natural Science, Seoul Woman's University, Seoul 139-774, Korea

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I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR : *Lactobacillus plantarum* PL 9-2	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY : KCCM - 10246

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

☐ a scientific description

☐ a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

the microorganism identified under I above was received by this international Depositary Authority on Dec. 2. 2000. and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on Jan. 31. 2001.

IV. INTERNATIONAL DEPOSITARY AUTHORITY

Name : Korean Culture Center of Microorganisms Address : 361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu SEOUL 120-091 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s) : Date: Jan. 31. 2001

Form BP/4

Next Page

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ATTESTATION CONCERNING THE LATER INDICATION OR AN
AMENDMENT OF THE SCIENTIFIC DESCRIPTION AND/OR
PROPOSED TAXONOMIC DESIGNATION

pursuant to Rule 8.2

TO Yeonhee lee
Department of Biology,
Seoul woman's University,
Seoul 139-774,
Korea

The enclosed communication has been received by this International Depositary Authority on Jun. 21. 2001.	
INDUSTRIAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : 361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu, SEOUL 120-091 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or authorized official(s) Date: Jun. 25. 2001.

Enclosure: Communication of the later indication or an amendment of the scientific description proposed taxonomic designation pursuant to Rule 8.1

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BUDAPEST TREATY ON THE INTERNATIONAL
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COMMUNICATION OF THE LATER INDICATION OR AN
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TO. KCCM
361-221 Yurim B/D
Hongje-1-dong Seodaemun-gu
Seoul 120-091
Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM

Accession number given by the
INTERNATIONAL DEOSITARY AUTHORITY:
KCCM 10246

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION ☐[1]

☐[2] Scientific description:

☐[2] Last preceding scientific description (if any):

☐[2] Proposed taxonomic designation:

Enterococcus PL 9002

☐[2] Last preceding proposed taxonomic designation (if any):

Lactobacillus CCARM 9-2

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III. REQUEST FOR ATTESTATION	
The undersigned [1] ☐[2] requests ☐[2] does not request the attestation referred to in Rule 8.2	
IV. DEPOSITOR	
Name: Yeonhee lee Address: Department of Biology, Seoul women's University, Seoul 139-774, Korea	Signature[3] :  Date: Jun. 21. 2001

[2]. Mark with a cross the applicable box.

[3]. Where the signature is required on behalf of a legal entity, the typewritten name(s) of the natural person(s) signing on the legal entity should accompany the signature(s).

Applicant's or agent's file reference	OPP010966KR	International application No.

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

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Address of depositary institution *(including postal code and country)*:

361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu
SEOUL, 120-091, Republic of Korea

Date of deposit	Accession Number
December 2, 2000	KCCM-10247

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

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Form PCT/RO/134 (July1998)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To. Yeon-hee Lee
Department of Biology and Culture Collection of Antibiotic Resistant Microbes, College of Natural Science, Seoul Woman's University, Seoul 139-774, Korea

RECEIPT IN THE CASE OF AN ORIGINAL
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR : *Lactobacillus paraparacasei* 1 PL 9-3	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY : KCCM - 10247

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

☐ a scientific description

☐ a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

the microorganism identified under I above was received by this international Depositary Authority on Dec. 2, 2000. and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on Jan. 31, 2001.

IV. INTERNATIONAL DEPOSITARY AUTHORITY

Name : Korean Culture Center of Microorganisms Address : 361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu SEOUL 120-091 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s) : Date: Jan. 31, 200

Form BP/4

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSE OF PATENT PROCEDURE

ATTESTATION CONCERNING THE LATER INDICATION OR AN
AMENDMENT OF THE SCIENTIFIC DESCRIPTION AND/OR
PROPOSED TAXONOMIC DESIGNATION

pursuant to Rule 8.2

TO Yeonhee lee
Department of Biology,
Seoul woman's University,
Seoul 139-774,
Korea

The enclosed communication has been received by this International Depositary Authority on Jun. 21. 2001.

INDUSTRIAL DEPOSITARY AUTHORITY

Name : Korean Culture Center of Microorganisms
Address : 361-221, Yurim B/D
Hongje-1-dong,
Seodaemun-gu,
SEOUL 120-091
Republic of Korea

Signature(s) of person(s) having the power to represent the International Depositary Authority or authorized official(s)

Date: Jun. 25. 2001.

Enclosure: Communication of the later indication or an amendment of the scientific description proposed taxonomic designation pursuant to Rule 8.1

Form BP/8 (sole page)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSE OF PATENT PROCEDURE

COMMUNICATION OF THE LATER INDICATION OR AN
AMENDMENT OF THE SCIENTIFIC DESCRIPTION
AND/OR PROPOSED TAXONOMIC DESIGNATION
pursuant to Rule 8.1

TO. KCCM
361-221 Yurim B/D
Hongje-1-dong Seodaemun-gu
Seoul. 120-091
Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM

Accession number given by the
INTERNATIONAL DEOSITARY AUTHORITY:
KCCM 10247

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION ☐[1]

☐[2] Scientific description:

☐[2] Last preceding scientific description (if any):

☐[2] Proposed taxonomic designation:

Enterococcus PL 9003

☐[2] Last preceding proposed taxonomic designation (if any):

Lactobacillus CCARM 9-3

[1]. Mark with a cross if additional information is given on an attached sheet.

[2]. Mark with a cross the applicable box or boxes.

Form BP/7 (first page)

III. REQUEST FOR ATTESTATION	
The undersigned [1]	
☐[3] requests	
☐[3] does not request	
the attestation referred to in Rule 8.2	
IV. DEPOSITOR	
Name: Yeonhee Lee	Signature[4] :
Address: Department of Biology, Seoul women's University, Seoul 139-774, Korea	Date: June. 21. 2001

[3]. Mark with a cross the applicable box.

[4]. Where the signature is required on behalf of a legal entity, the typewritten name(s) of the natural person(s) signing on the legal entity should accompany the signature(s).

Applicant's or agent's file reference	International application No.
OPP010966KR	

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 8, line 3.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution
KOREA CULTURE CENTER OF MICROORGANISMS

Address of depositary institution *(including postal code and country)*
361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu
SEOUL, 120-091, Republic of Korea

Date of deposit	Accession Number
December 2, 2000	KCCM-10248

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☑ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

Form PCT/RO/134 (July1998)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To. Yeon-hee Lee
Department of Biology and Culture Collection of Antibiotic Resistant Microbes, College of Natural Science, Seoul Woman's University, Seoul 139-774, Korea

RECEIPT IN THE CASE OF AN ORIGINAL issued pursuant to Rule 7.1 by the INTERNATIONAL DEPOSITARY AUTHORITY identified at the bottom of this page

I .IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR : *Lactobacillus coprophilus* PL 9-4	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY : KCCM - 10248

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

☐ a scientific description

☐ a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

the microorganism identified under I above was received by this international Depositary Authority on Dec. 2. 2000. and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on Jan. 31. 2001.

IV. INTERNATIONAL DEPOSITARY AUTHORITY

Name : Korean Culture Center of Microorganisms Address : 361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu SEOUL 120-091 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s) : Date: Jan. 31. 200

Form BP/4 Sole page

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSE OF PATENT PROCEDURE

ATTESTATION CONCERNING THE LATER INDICATION OR AN
AMENDMENT OF THE SCIENTIFIC DESCRIPTION AND/OR
PROPOSED TAXONOMIC DESIGNATION

pursuant to Rule 8.2

TO Yeonhee lee
Department of Biology,
Seoul woman's University,
Seoul 139-774,
Korea

The enclosed communication has been received by this International Depositary Authority on Jun. 21. 2001.

INDUSTRIAL DEPOSITARY AUTHORITY

Name : Korean Culture Center of Microorganisms
Address : 361-221, Yurim B/D
Hongje-1-dong,
Seodaemun-gu,
SEOUL 120-091
Republic of Korea

Signature(s) of person(s) having the power to represent the International Depositary Authority or authorized official(s) :

Date: Jun. 25. 2001.

Enclosure: Communication of the later indication or an amendment of the scientific description proposed taxonomic designation pursuant to Rule 8.1

Form BP/8 (sole page)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSE OF PATENT PROCEDURE

COMMUNICATION OF THE LATER INDICATION OR AN
AMENDMENT OF THE SCIENTIFIC DESCRIPTION
AND/OR PROPOSED TAXONOMIC DESIGNATION
pursuant to Rule 8.1

TO. KCCM
361-221 Yurim B/D
Hongje-1-dong Seodaemun-gu
Seoul. 120-091
Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM

Accession number given by the
INTERNATIONAL DEOSITARY AUTHORITY:
KCCM 10248

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION ☐[1]

☐[2] Scientific description:

☐[2] Last preceding scientific description (if any):

☐[2] Proposed taxonomic designation:

Lactobacillus PL 9004

☐[2] Last preceding proposed taxonomic designation (if any):

Lactobacillus CCARM 9-4

[1]. Mark with a cross if additional information is given on an attached sheet.

[2]. Mark with a cross the applicable box or boxes.

Form BP/7 (first page)

III. REQUEST FOR ATTESTATION	
The undersigned [1] ☐[3] requests ☐[3] does not request the attestation referred to in Rule 8.2	
IV. DEPOSITOR	
Name: Yeonhee lee Address: Department of Biology, Seoul women's University, Seoul 139-774, Korea	Signature[4] :  Date: June. 21. 2001

[3]. Mark with a cross the application box.

[4]. Where the signature is required on behalf of a legal entity, the typewritten name(s) of the natural person(s) signing on the legal entity should accompany the signature(s).

Applicant's or agent's file reference	OPP010966KR	International application No.

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 8, line 4.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution

KOREA CULTURE CENTER OF MICROORGANISMS

Address of depositary institution *(including postal code and country)*

361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu
SEOUL, 120-091, Republic of Korea

Date of deposit	Accession Number
March 2, 2001	KCCM-10250

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☑ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

Form PCT/RO/134 (July 1998)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To. Yeonhee Lee
Department of Biology,
Seoul Woman's University,
Seoul 139-774, Korea

RECEIPT IN THE CASE OF AN ORIGINAL
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR : *Lactobacillus* PL 9-5	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM-10250
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on Mar. 2, 2001. (date of the original deposit)[1]	
IV. INTERNATIONAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : 361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu SEOUL 120-091 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority of of authouized official(s): Date: Mar. 8, 2001.

1 Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired : where a deposit made outside the Budapest Treaty after the acquisition of the status of international depositary authority is converted into a deposit under the Budapest Treaty, such date is the date on which the microorganism was received by the international depositary authority.

Form BP/4 Sole page

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSE OF PATENT PROCEDURE

ATTESTATION CONCERNING THE LATER INDICATION OR AN
AMENDMENT OF THE SCIENTIFIC DESCRIPTION AND/OR
PROPOSED TAXONOMIC DESIGNATION

pursuant to Rule 8.2

TO Yeonhee Lee
Department of Biology,
Seoul woman's University,
Seoul 139-774,
Korea

The enclosed communication has been received by this International Depositary Authority on Jun. 21. 2001.	
INDUSTRIAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : 361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu, SEOUL 120-091 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or authorized official(s) : Date: Jun. 25. 2001.

Enclosure: Communication of the later indication or an amendment of the scientific description proposed taxonomic designation pursuant to Rule 8.1

Form BP/8 (sole page)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSE OF PATENT PROCEDURE

COMMUNICATION OF THE LATER INDICATION OR AN
AMENDMENT OF THE SCIENTIFIC DESCRIPTION
AND/OR PROPOSED TAXONOMIC DESIGNATION
pursuant to Rule 8.1

TO. KCCM
361-221 Yurim B/D
Hongje-1-dong Seodaemun-gu
Seoul. 120-091
Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM

Accession number given by the
INTERNATIONAL DEOSITARY AUTHORITY:
KCCM 10250

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION ☐[1]

☐[2] Scientific description:

☐[2] Last preceding scientific description (if any):

☐[2] Proposed taxonomic designation:

Lactobacillus PL 9005

☐[2] Last preceding proposed taxonomic designation (if any):

Lactobacillus PL 9-5

[1]. Mark with a cross if additional information is given on an attached sheet.

[2]. Mark with a cross the applicable box or boxes.

Form BP/7 (first page)

III. REQUEST FOR ATTESTATION	
The undersigned 111 ☐[3] requests ☐[3] does not request the attestation referred to in Rule 8.2	
IV. DEPOSITOR	
Name: Yeonhee lee Address: Department of Biology, Seoul women's University, Seoul 139-774, Korea	Signature[4] :  Date: June. 21. 2001

[3]. Mark with a cross the application box.

[4]. Where the signature is required on behalf of a legal entity, the typewritten name(s) of the natural person(s) signing on the legal entity should accompany the signature(s).

Applicant's or agent's file reference	International application No.
OPP010966KR	

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 8, line 5.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution: KOREA CULTURE CENTER OF MICROORGANISMS

Address of depositary institution *(including postal code and country)*:

361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu
SEOUL, 120-091, Republic of Korea

Date of deposit	Accession Number
March, 2, 2001	KCCM-10251

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☑ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

Form PCT/RO/134 (July1998)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To. Yeonhee Lee
Department of Biology,
Seoul Woman's University,
Seoul 139-774, Korea

RECEIPT IN THE CASE OF AN ORIGINAL
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR : *Lactobacillus* PL 9-6	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM-10251
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on Mar. 2. 2001. (date of the original deposit)[1]	
IV. INTERNATIONAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : 361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu SEOUL 120-091 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority of of authoulzed official(s) : Date: Mar. 8. 2001.

1 Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired : where a deposit made outside the Budapest Treaty after the acquisition of the status of International depositary authority is converted into a deposit under the Budapest Treaty, such date is the date on which the microorganism was received by the international depositary authority.

Form BP/4 Sole page

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSE OF PATENT PROCEDURE

ATTESTATION CONCERNING THE LATER INDICATION OR AN
AMENDMENT OF THE SCIENTIFIC DESCRIPTION AND/OR
PROPOSED TAXONOMIC DESIGNATION

pursuant to Rule 8.2

TO Yeonbee lee
Department of Biology,
Seoul woman's University,
Seoul 139-774,
Korea

The enclosed communication has been received by this International Depositary Authority on Jun. 21. 2001.	
INDUSTRIAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : 361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu, SEOUL 120-091 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or authorized official(s) : Date: Jun. 25. 2001.

Enclosure: Communication of the later indication or an amendment of the scientific description proposed taxonomic designation pursuant to Rule 8.1

Form BP/8 (sole page)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSE OF PATENT PROCEDURE

COMMUNICATION OF THE LATER INDICATION OR AN
AMENDMENT OF THE SCIENTIFIC DESCRIPTION
AND/OR PROPOSED TAXONOMIC DESIGNATION
pursuant to Rule 8.1

TO. KCCM
361-221 Yurim B/D
Hongje-1-dong Seodaemun-gu
Seoul. 120-091
Republic of Korea

I. IDENTIFICATION OF THE MICROORGANISM

Accession number given by the
INTERNATIONAL DEOSITARY AUTHORITY:
KCCM 10251

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION ☐[1]

☐[2] Scientific description:

☐[2] Last preceding scientific description (if any):

☐[2] Proposed taxonomic designation:

Lactobacillus PL 9006

☐[2] Last preceding proposed taxonomic designation (if any):

Lactobacillus PL 9-6

[1]. Mark with a cross if additional information is given on an attached sheet.

[2]. Mark with a cross the applicable box or boxes.

Form BP/7 (first page)

III. REQUEST FOR ATTESTATION	
The undersigned ☐[3] requests ☐[3] does not request the attestation referred to in Rule 8.2	
IV. DEPOSITOR	
Name: Yeonhee lee Address: Department of Biology, Seoul women's University, Seoul 139-774, Korea	Signature[4] :  Date: June. 21. 2001

[3]. Mark with a cross the application box.

[4]. Where the signature is required on behalf of a legal entity, the typewritten name(s) of the natural person(s) signing on the legal entity should accompany the signature(s).

WHAT IS CLAIMED IS:

1. A bacterium for inhibiting the adhesion of *Helicobacter pylori* to the gastric mucosa, wherein the bacterium is at least one selected from the group consisting of *Lactobacillus coprophilus, Enterococcus durans, Streptococcus faecalis*, and *Lactobacillus fermentum*.

2. The bacterium of claim 1, wherein the bacterium is at least one selected from the group consisting of *Lactobacillus coprophilus* PL 9001 (KCCM-10245), *Enterococcus durans* PL 9002 (KCCM-10246), *Streptococcus faecalis* PL 9003 (KCCM-10247), *Lactobacillus coprophilus* PL 9004 (KCCM-10248), *Lactobacillus fermentum* PL 9005 (KCCM-10250), and *Lactobacillus fermentum* PL 9006 (KCCM-10251).

3. The bacterium of claim 1, wherein bacterium is live, dehydrated, or non-viable bacterium.

4. A bacterium for inhibiting the growth of *Helicobacter pylori*, wherein the bacterium is at least one selected from the group consisting of *Lactobacillus coprophilus, Enterococcus durans, Streptococcus faecalis*, and *Lactobacillus fermentum*.

5. The bacterium of claim 4, wherein the bacterium is at least one selected from the group consisting of *Lactobacillus coprophilus* PL 9001 (KCCM-10245), *Enterococcus durans* PL 9002 (KCCM-10246), *Streptococcus faecalis* PL 9003 (KCCM-10247), *Lactobacillus coprophilus* PL 9004 (KCCM-10248), *Lactobacillus fermentum* PL 9005 (KCCM-10250),

and *Lactobacillus fermentum* PL 9006 (KCCM-10251).

6. A composition for inhibiting the growth of bacteria, wherein the composition comprises at least one bacterium selected from the group consisting of *Lactobacillus coprophilus, Enterococcus durans, Streptococcus faecalis*, and *Lactobacillus fermentum*.

7. The composition of claim 6, wherein the composition comprises at least one selected from the group consisting of *Lactobacillus coprophilus* PL 9001 (KCCM-10245), *Enterococcus durans* PL 9002 (KCCM-10246), *Streptococcus faecalis* PL 9003 (KCCM-10247), *Lactobacillus coprophilus* PL 9004 (KCCM-10248), *Lactobacillus fermentum* PL 9005 (KCCM-10250), and *Lactobacillus fermentum* PL 9006 (KCCM-10251).

8. The composition of claim 6, wherein the bacteria is *Helicobacter pylori*, a bacteria that causes food poisoning, a bacteria that causes acne, or anaerobic bacteria.

9. The composition of claim 6, wherein the bacterium is a live bacterium, fragments of cell walls of the same, or culture filtrate of the same.

10. A cosmetic composition comprising at least one bacterium selected from the group consisting of *Lactobacillus coprophilus, Enterococcus durans, Streptococcus faecalis*, *Lactobacillus fermentum*, and a culture filtrate of bacteria thereof.

11. The cosmetic composition of claim 10, wherein the bacterium is at least one selected from the group consisting of *Lactobacillus*

coprophilus PL 9001 (KCCM-10245), *Enterococcus durans* PL 9002 (KCCM-10246), *Streptococcus faecalis* PL 9003(KCCM-10247), *Lactobacillus coprophilus* PL 9004 (KCCM-10248), *Lactobacillus fermentum* PL 9005 (KCCM-10250, and *Lactobacillus fermentum* PL 9006 (KCCM-10251).

12. The cosmetic composition of claim 10, wherein the cosmetic composition is for external application against infectious disease.

13. The cosmetic composition of claim 10, wherein the cosmetic composition is for treatment of acne.

14. A food additive comprising at least one bacterium selected from the group consisting of *Lactobacillus coprophilus, Enterococcus durans, Streptococcus faecalis*, *Lactobacillus fermentum*, and a culture filtrate of bacteria thereof.

15. The food additive of claim 14, wherein the food additive is applied to yogurt, weaning diet, dairy goods, cheese, Kimchi, drinks, or solid food.

16. A food prepared by fermenting at least one bacterium selected from the group consisting of *Lactobacillus coprophilus, Enterococcus durans, Streptococcus faecalis*, and *Lactobacillus fermentum*.

17. An immunological enhancement composition comprising at least one bacterium selected from the group consisting of *Lactobacillus coprophilus, Enterococcus durans, Streptococcus faecalis*, *Lactobacillus fermentum*, and a culture filtrate of bacteria thereof.

18. The composition of claim 17, wherein the composition induces expression of tumor necrosis factor-α or interlukin-6.

19. A composition for curing intestinal disorders comprising at least one bacteria selected from the group consisting of *Lactobacillus coprophilus, Enterococcus durans, Streptococcus faecalis*, *Lactobacillus fermentum*, and a culture filtrate of bacteria thereof.

20. The composition of claim 19, wherein the composition inhibits the growth of at least one bacterium selected from the group consisting of *Helicobacter pylori*, a bacillus that causes food poisoning, and anaerobic bacteria.

Fig. 1

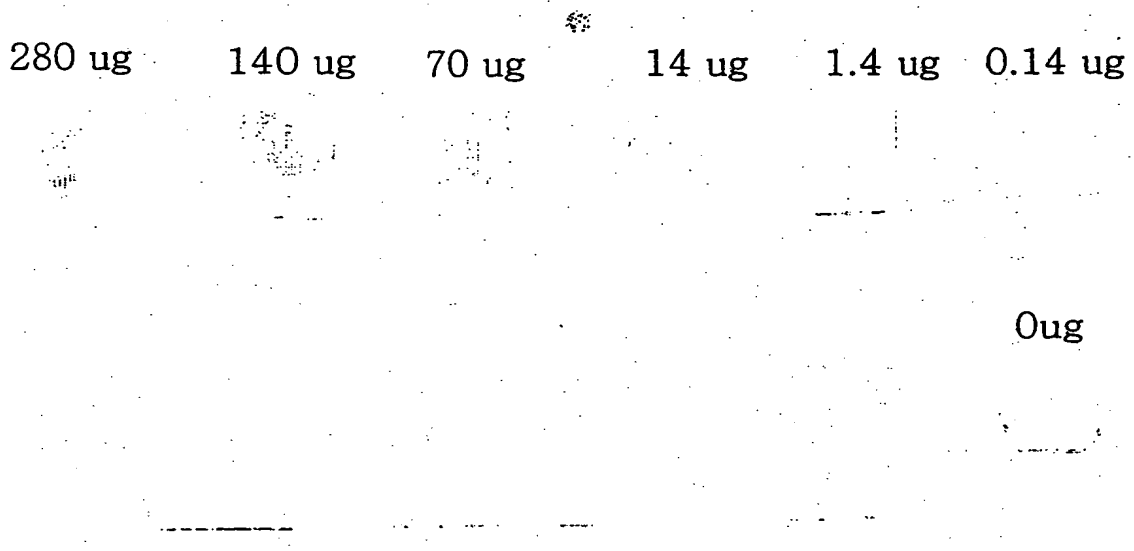


Fig. 2

Control	Competitive Lipid Binding Assay		
Lipid	PL9001	PL9002	PL9003
Helicobacter pylori	PL9004	PL9005	PL9006

Fig. 3a

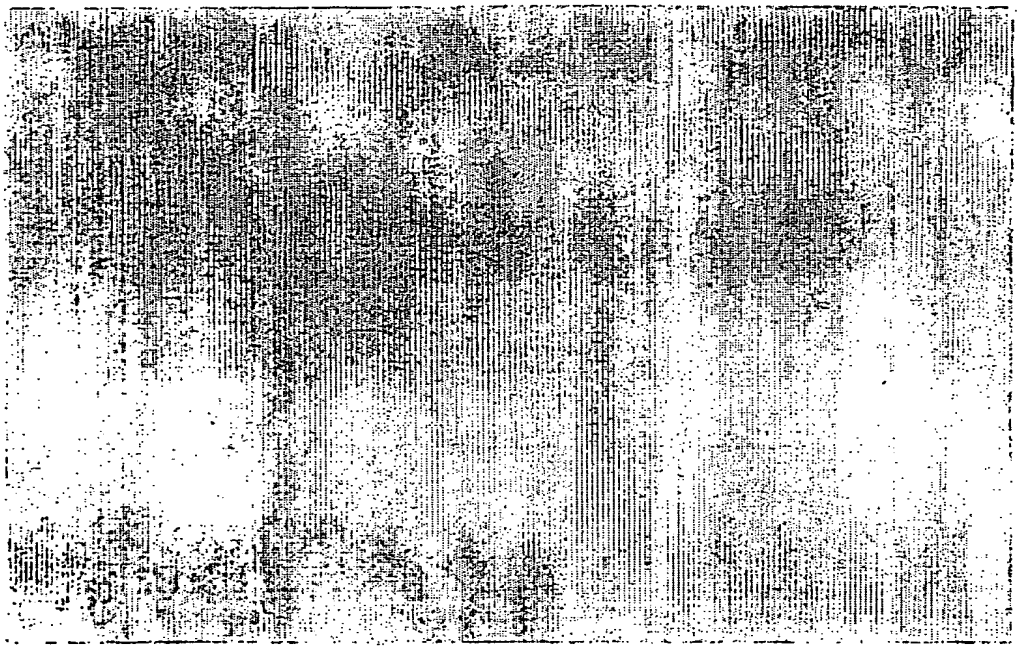


Fig. 3b

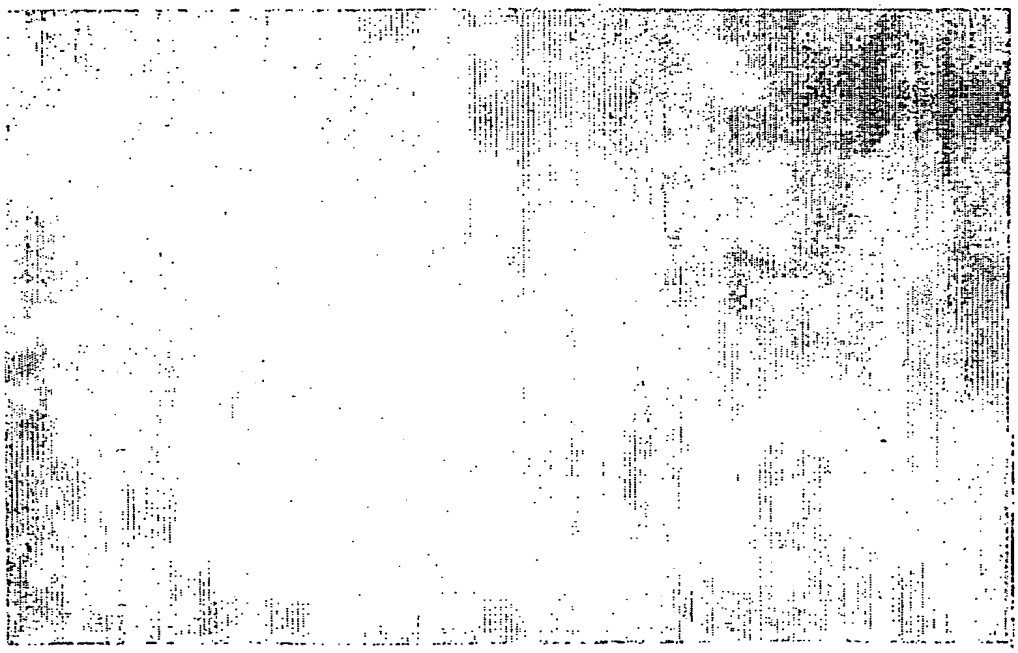


Fig. 3c

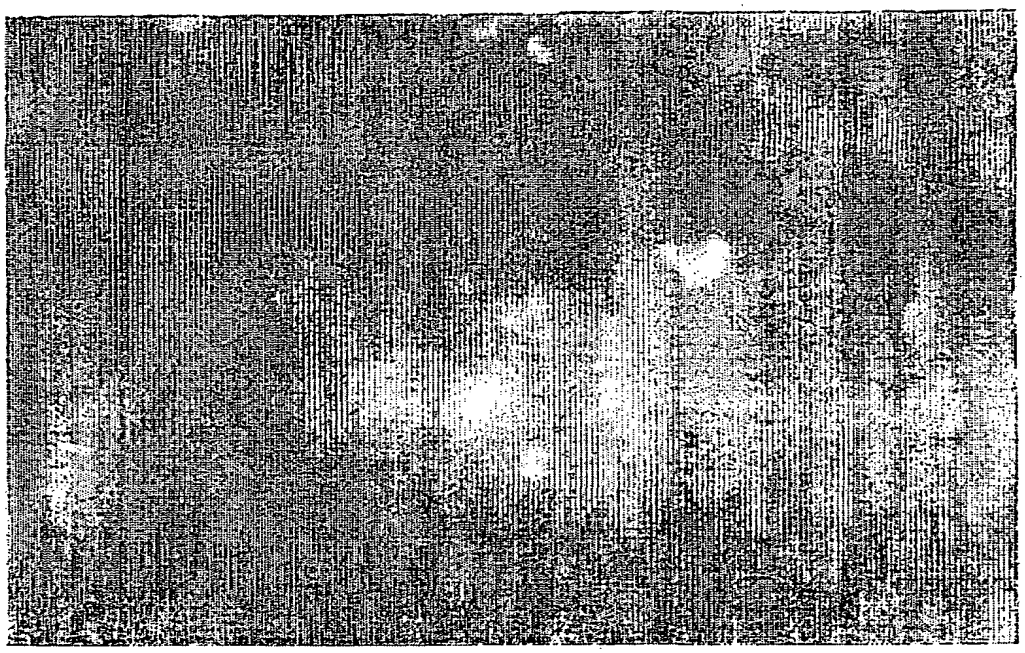


Fig. 3d

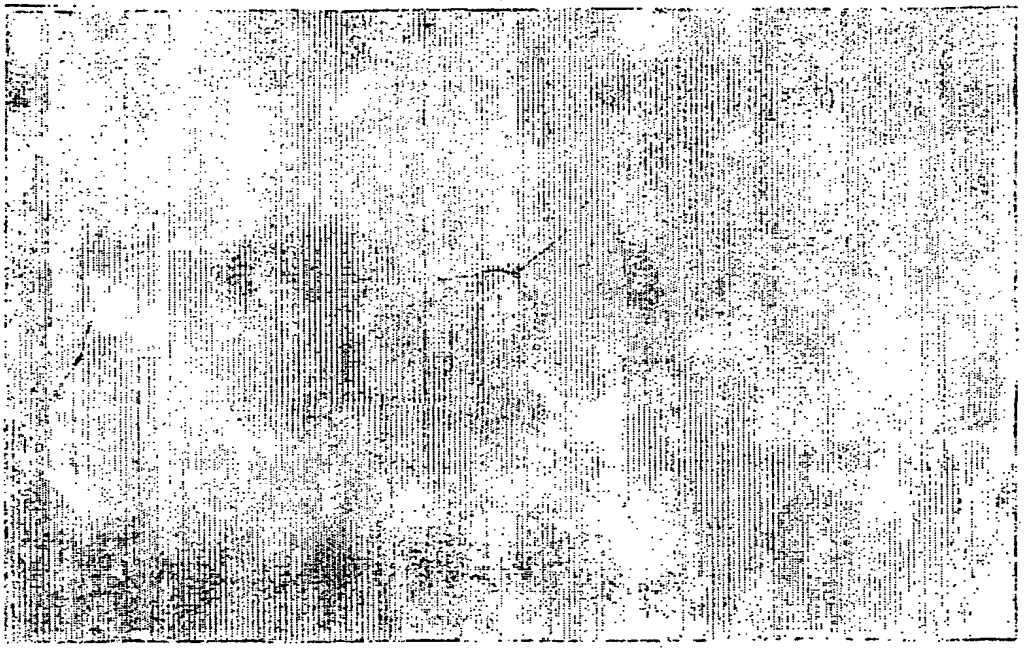


Fig. 3e

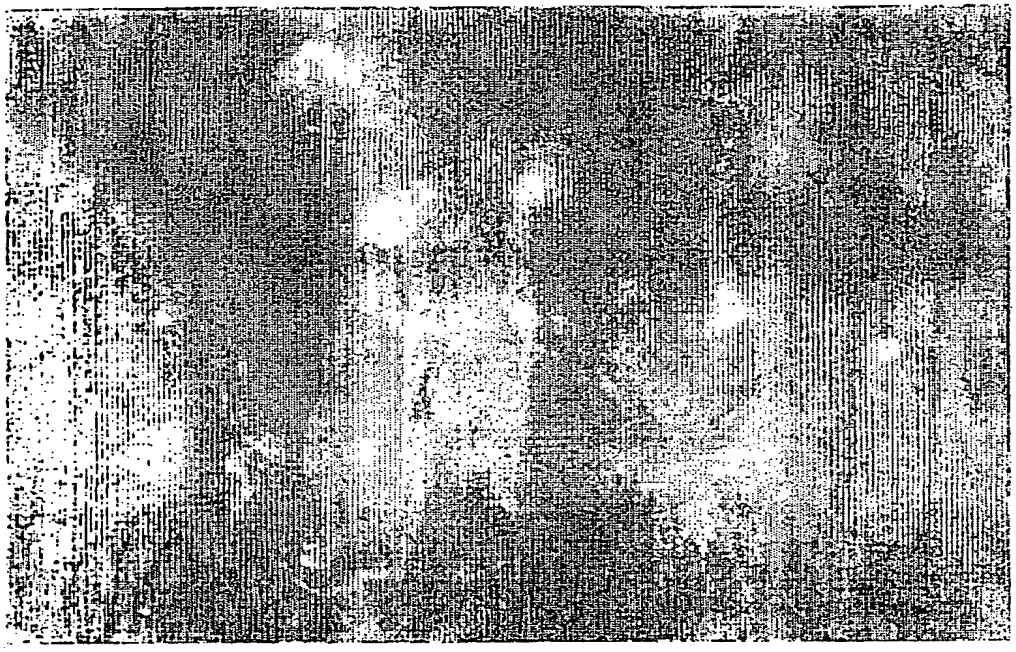


Fig. 3f

Fig. 3g

Fig. 3h

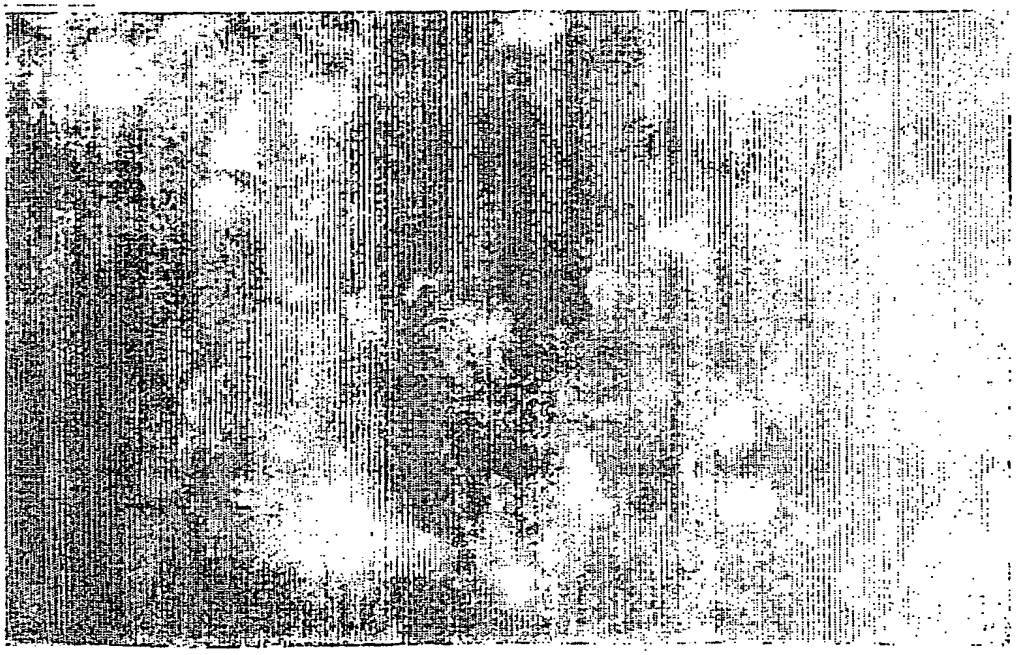


Fig. 3i

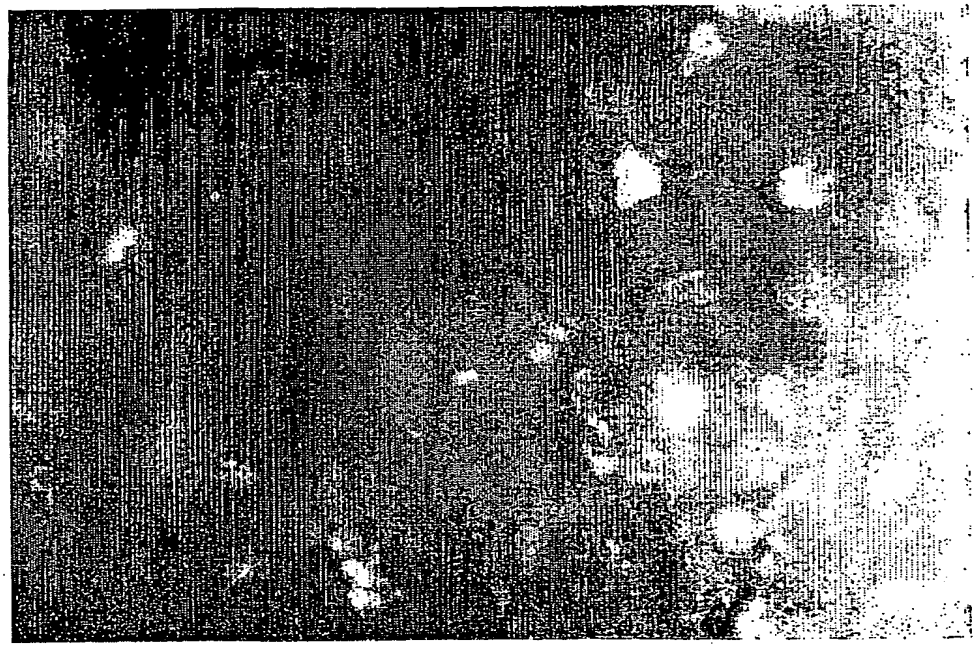


Fig. 3j

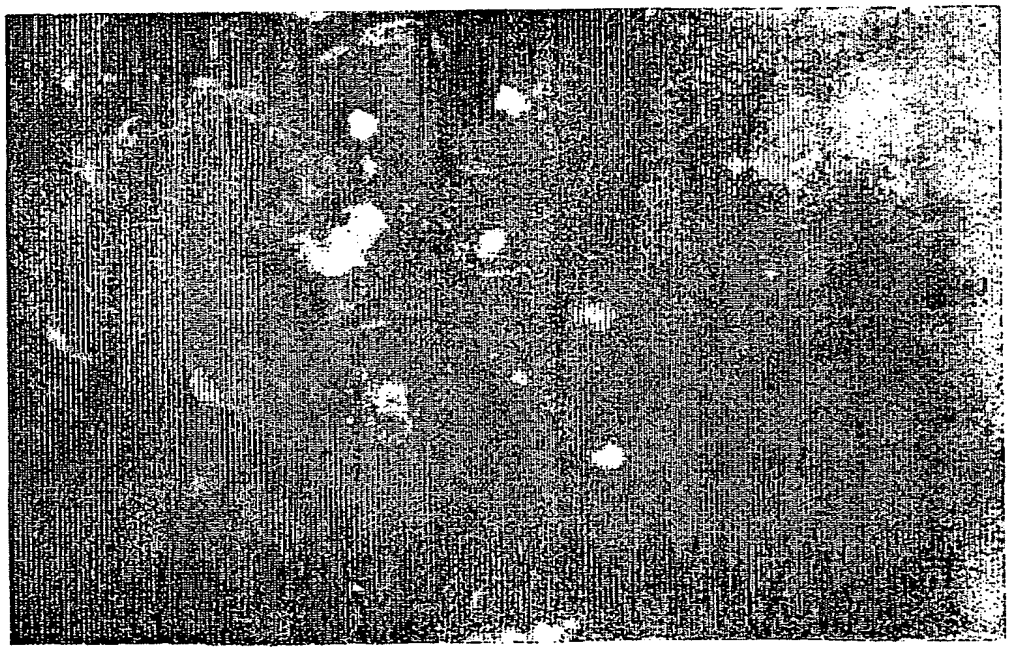


Fig. 3k

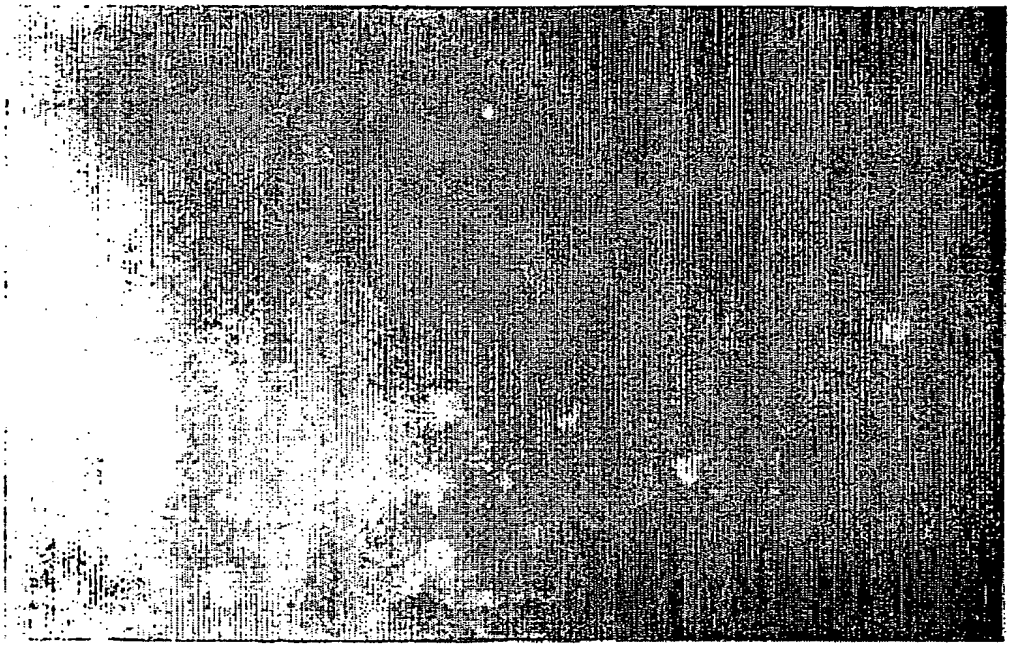


Fig. 3l

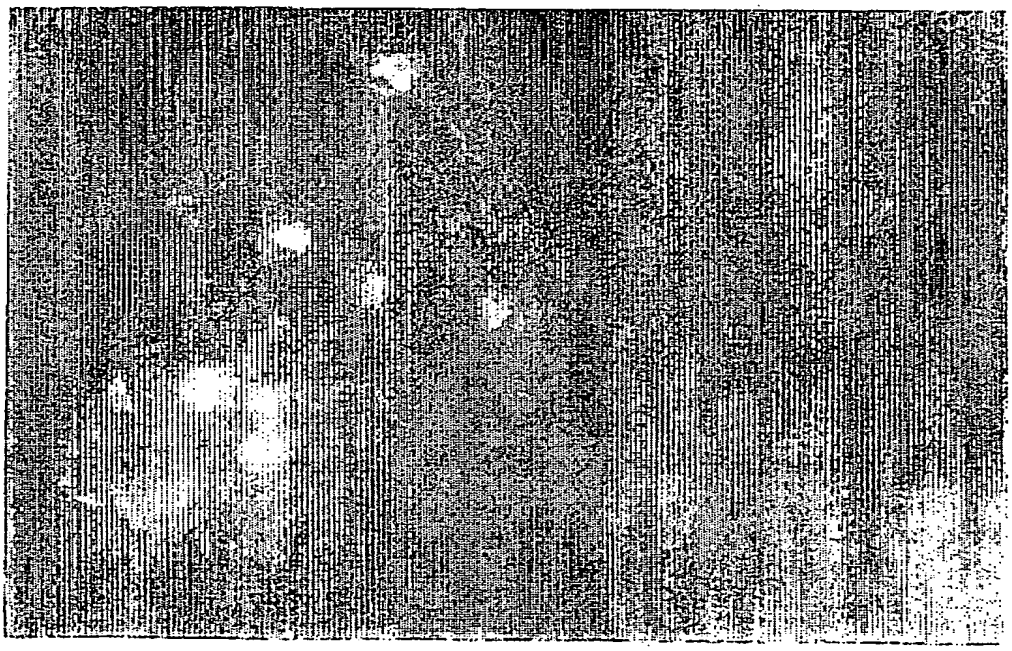


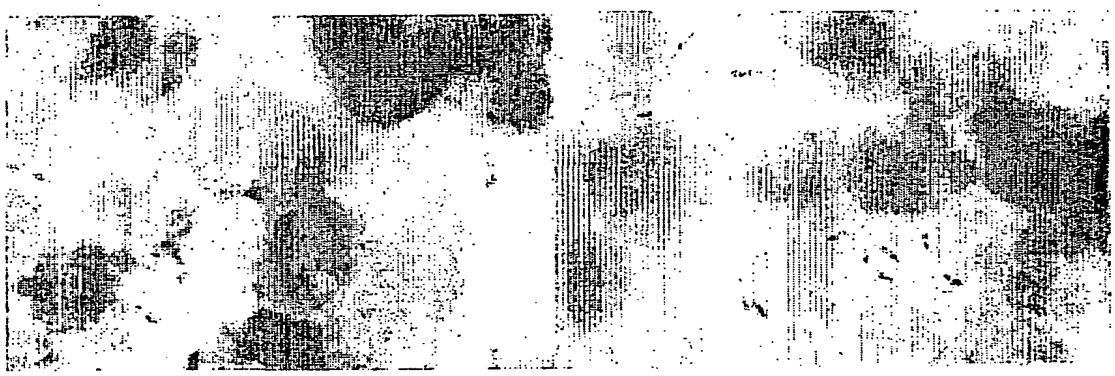
Fig. 4a

(a) (a')

Fig. 4b

(b) (b')

Fig. 4c



(c) (c')

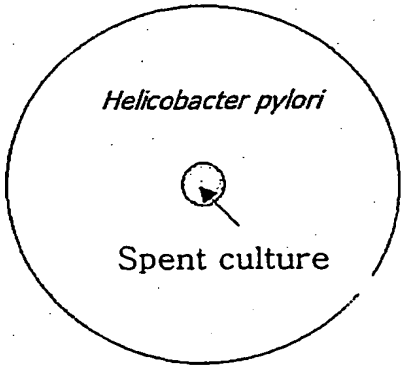
Fig. 4d

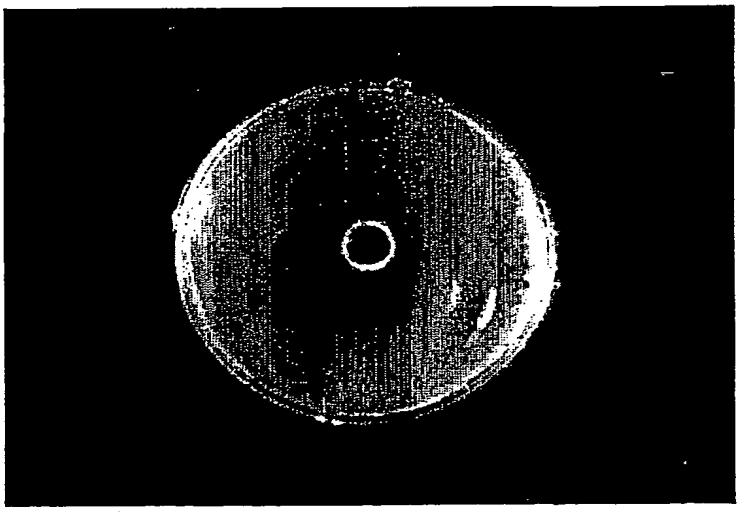
(d) (d')

Fig. 4e

(e) (e')

Fig. 5





Diameter of Growth-Inhibited-zone

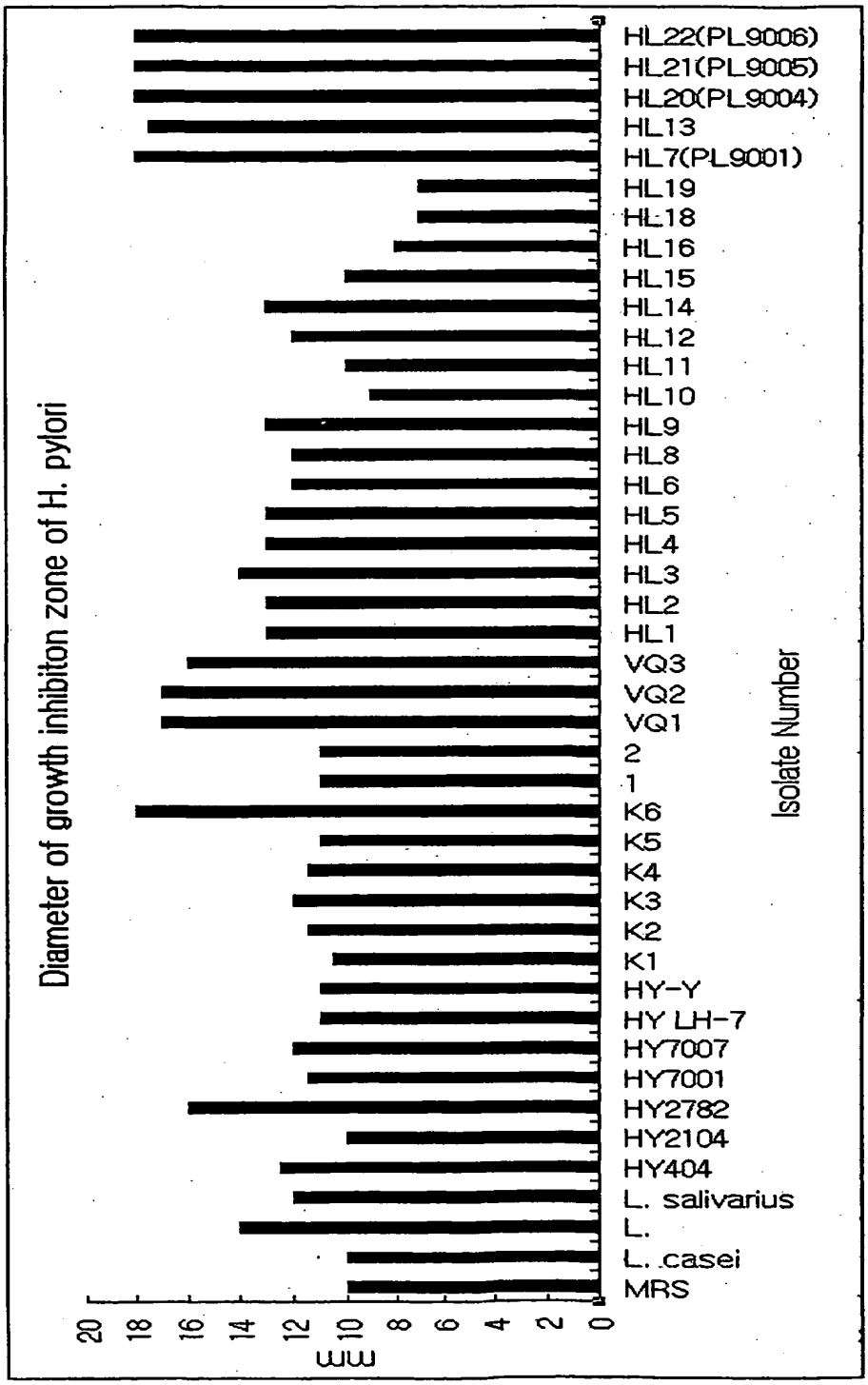

Diameter of growth inhibiton zone of H. pylori
mm
20
18
16
14
12
10
8
6
4
2
0
Isolate Number
HL22(PL9006)
HL21(PL9005)
HL20(PL9004)
HL13
HL7(PL9001)
HL19
HL18
HL16
HL15
HL14
HL12
HL11
HL10
HL9
HL8
HL6
HL5
HL4
HL3
HL2
HL1
VQ3
VQ2
VQ1
2
1
K6
K5
K4
K3
K2
K1
HY-Y
HY LH-7
HY7007
HY7001
HY2782
HY2104
HY404
L. salivarius
L.
L. casei
MRS

Fig. 6

Fig. 7

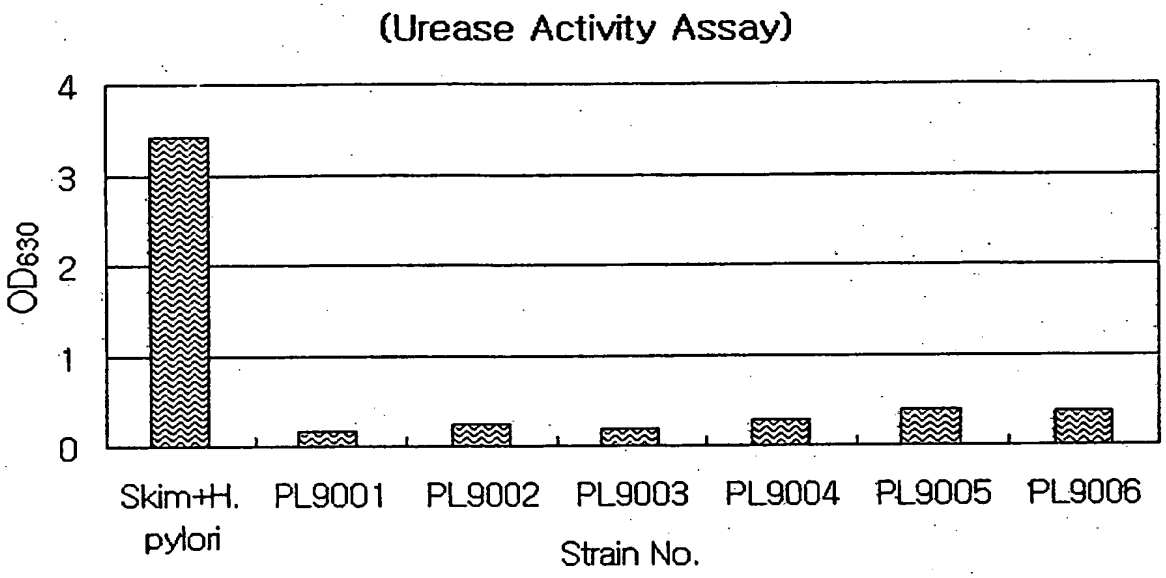

(Urease Activity Assay)
OD630
4
3
2
1
0
Skim+H. pylori
PL9001
PL9002
PL9003
PL9004
PL9005
PL9006
Strain No.

Fig. 8a

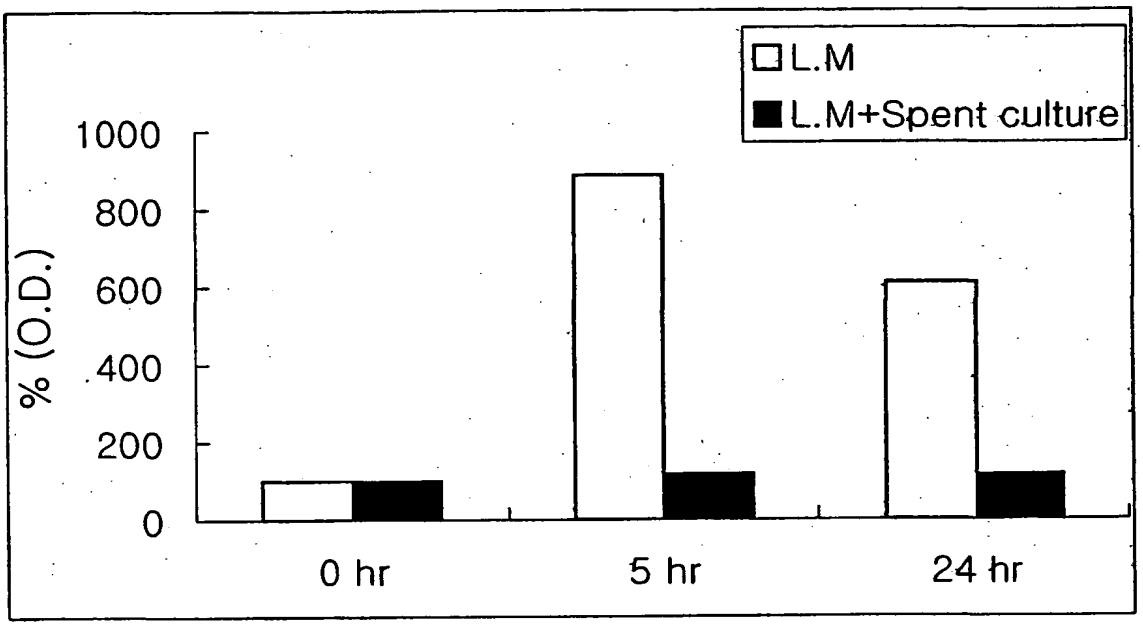

L.M
L.M+Spent culture
1000
800
600
400
200
0
% (O.D.)
0 hr
5 hr
24 hr

Fig. 8b

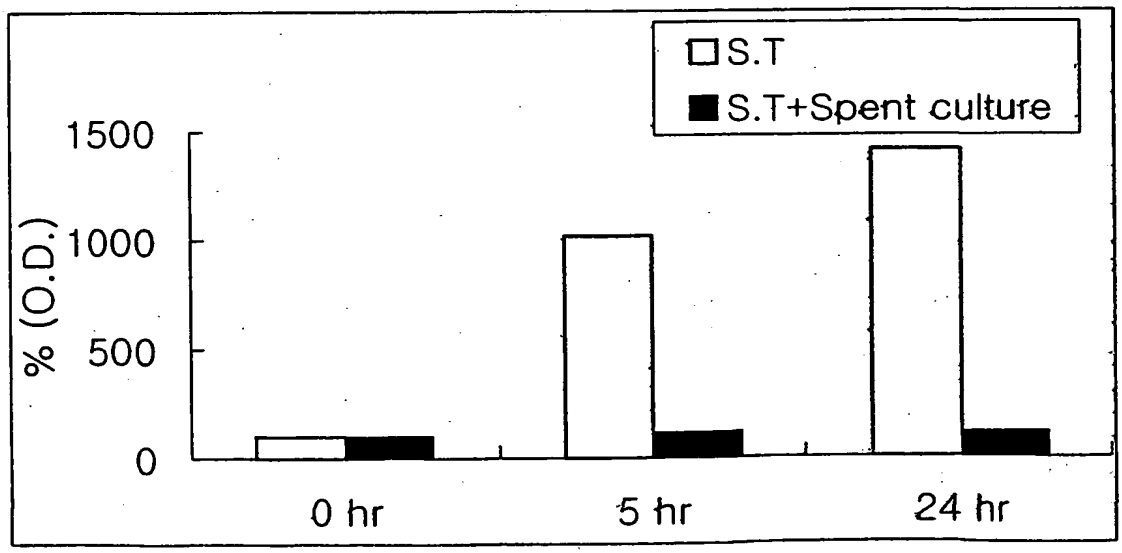

S.T
S.T+Spent culture
1500
1000
500
0
% (O.D.)
0 hr
5 hr
24 hr

Fig. 8c

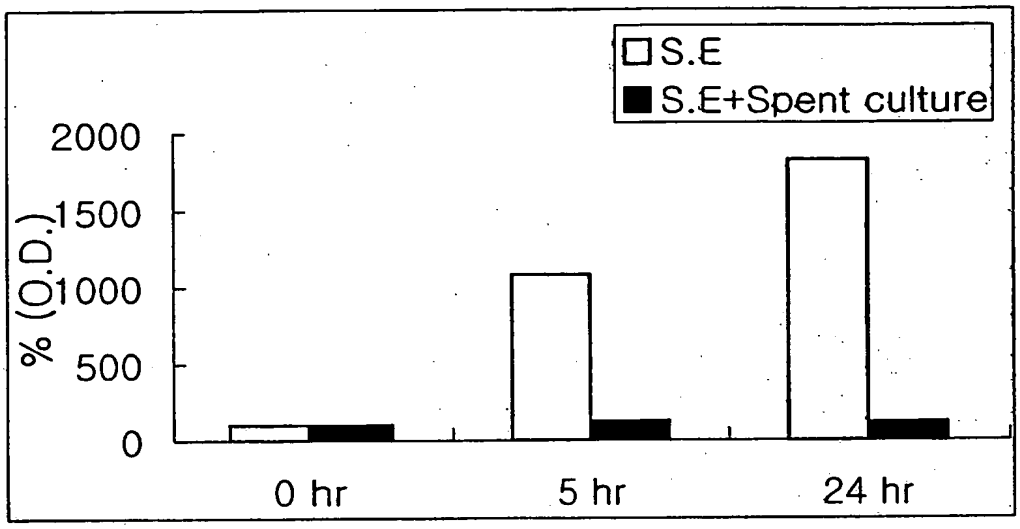

S.E
S.E+Spent culture
2000
1500
1000
500
0
% (O.D.)
0 hr
5 hr
24 hr

Fig. 8d

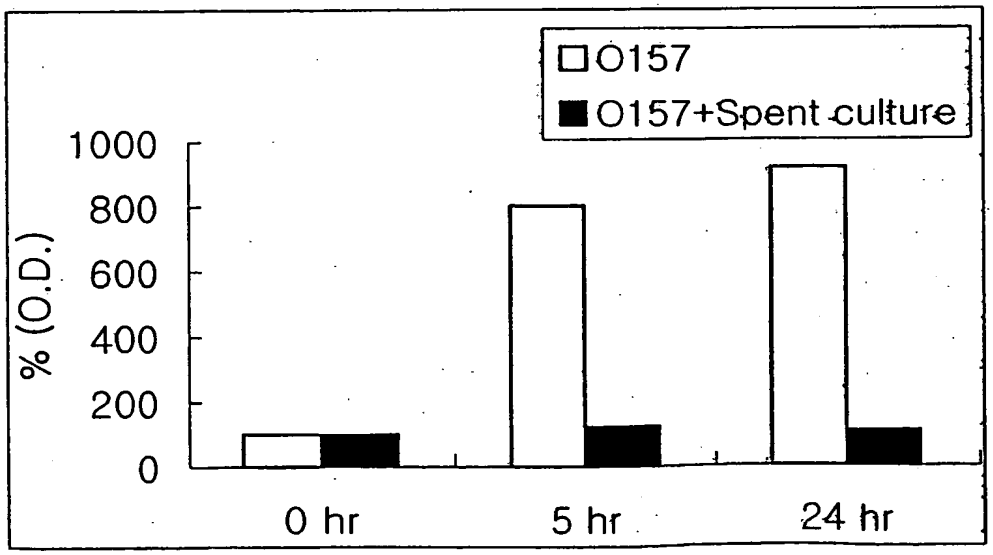

O157
O157+Spent culture
1000
800
600
400
200
0
% (O.D.)
0 hr
5 hr
24 hr

Fig. 8e

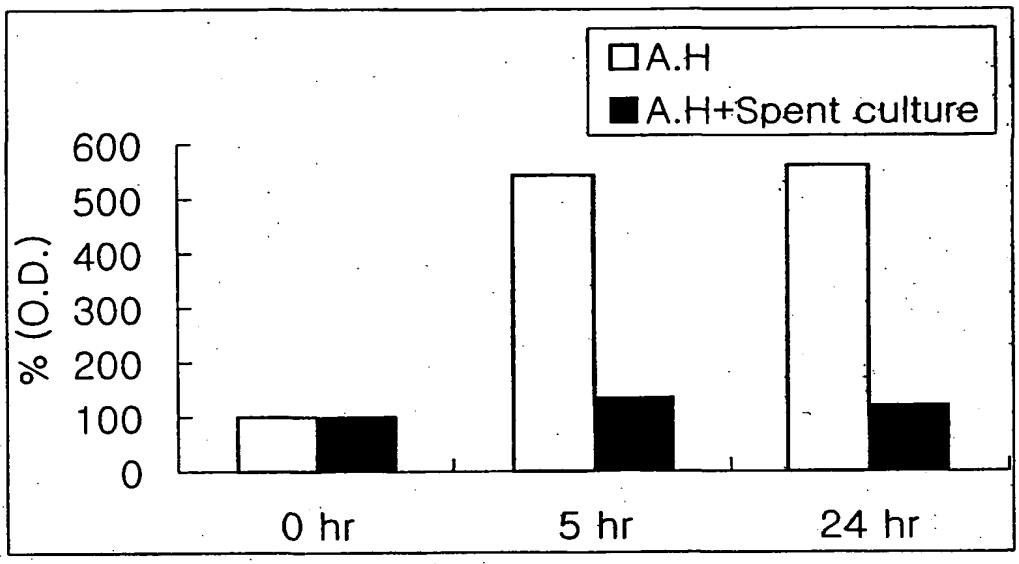

A.H
A.H+Spent culture
600
500
400
300
200
100
0
% (O.D.)
0 hr
5 hr
24 hr

Fig. 9a

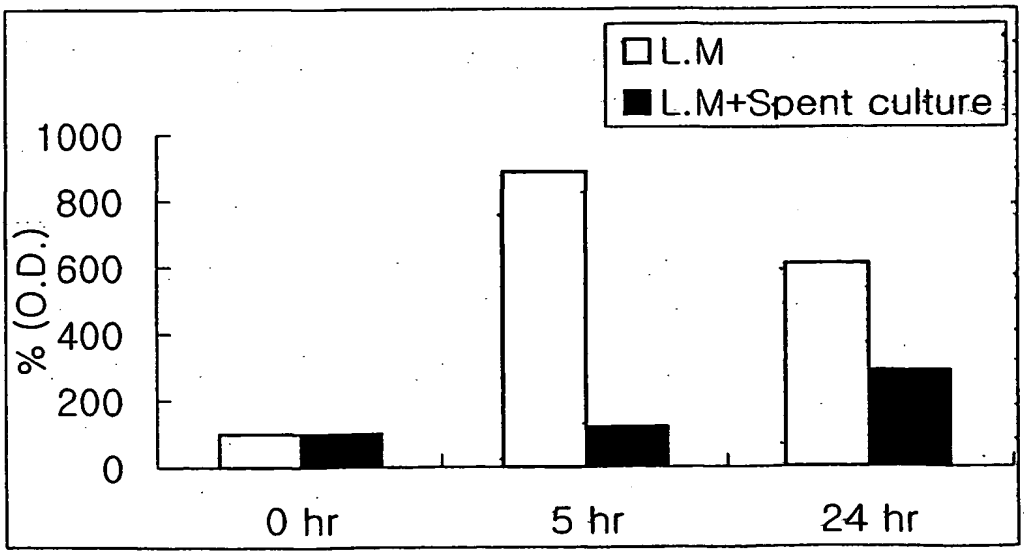

L.M
L.M+Spent culture
1000
800
600
400
200
0
% (O.D.)
0 hr
5 hr
24 hr

Fig. 9b

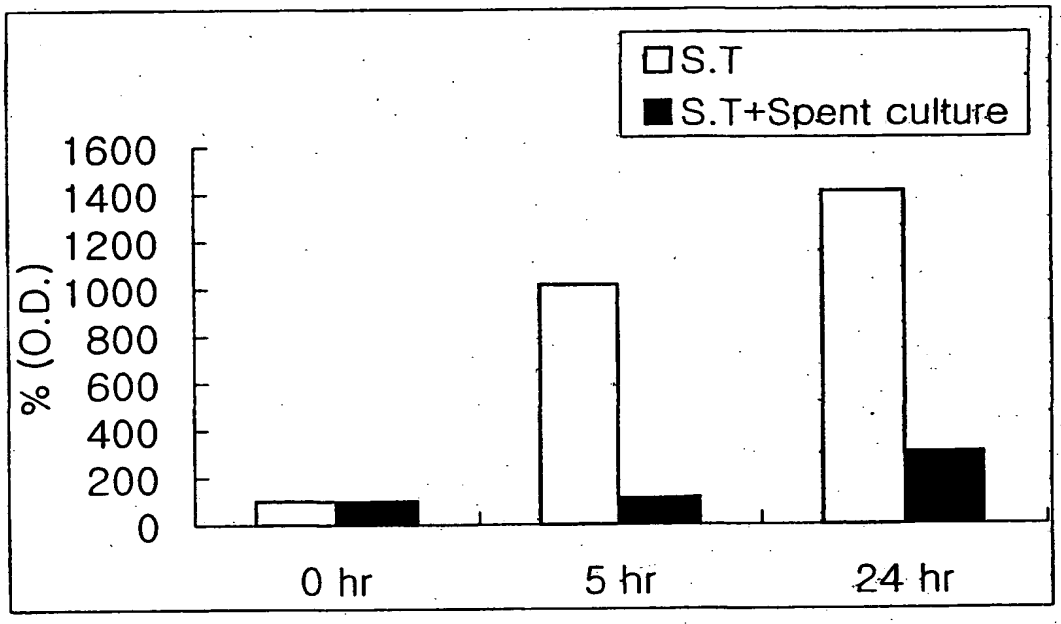

S.T
S.T+Spent culture
% (O.D.)
1600
1400
1200
1000
800
600
400
200
0
0 hr
5 hr
24 hr

Fig. 9c

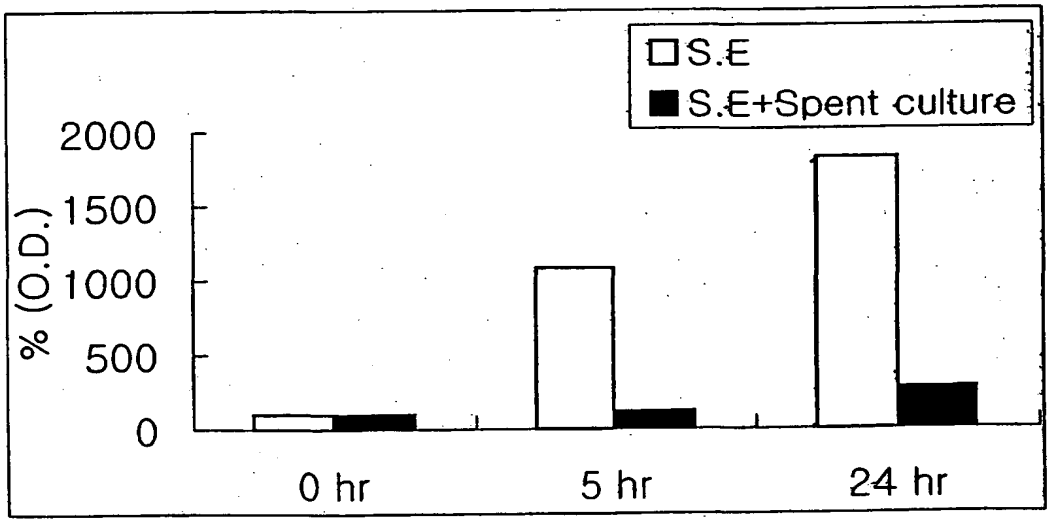

S.E
S.E+Spent culture
% (O.D.)
2000
1500
1000
500
0
0 hr
5 hr
24 hr

Fig. 9d

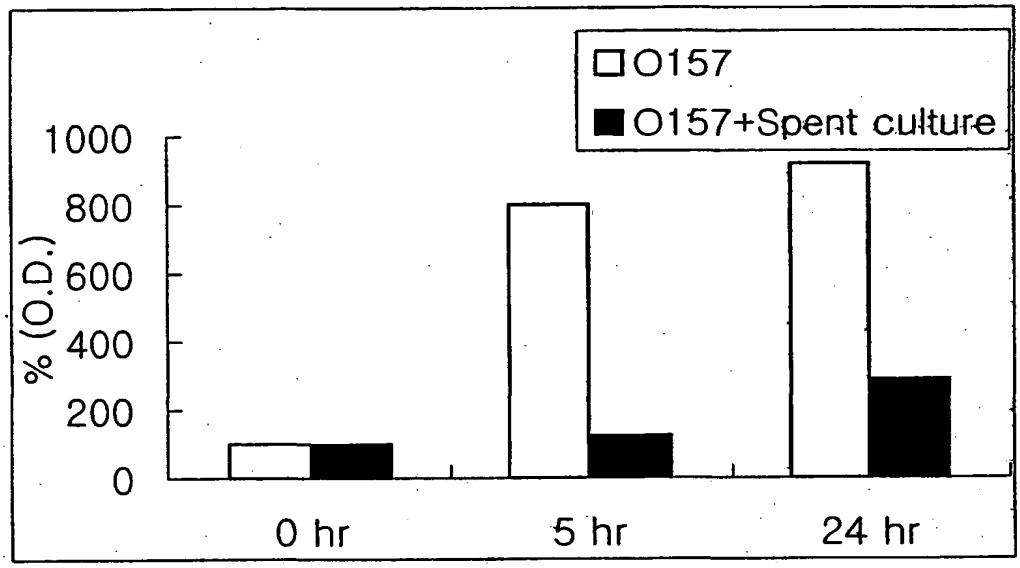

O157
O157+Spent culture
1000
800
600
400
200
0
% (O.D.)
0 hr
5 hr
24 hr

Fig. 9e

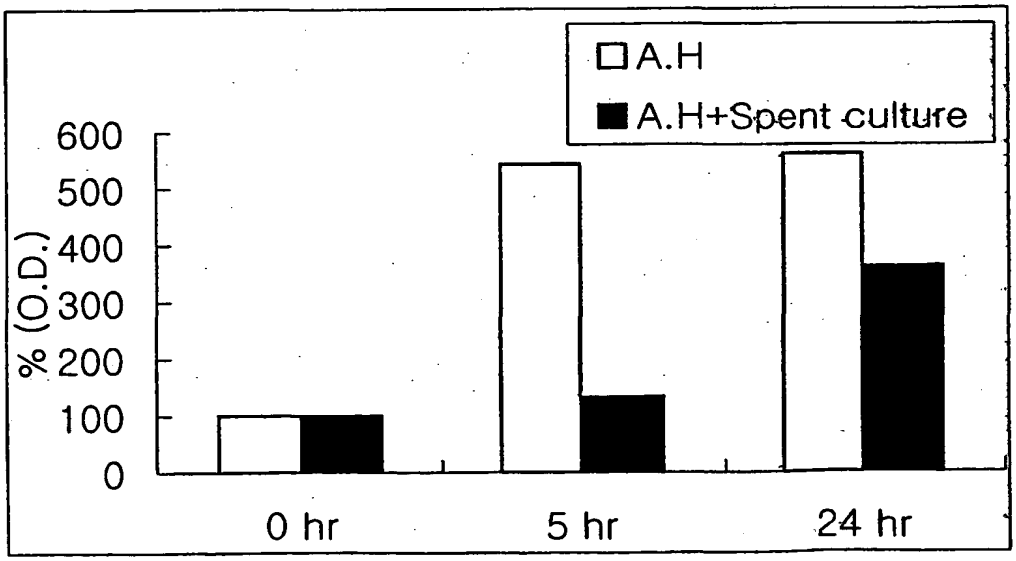

A.H
A.H+Spent culture
600
500
400
300
200
100
0
% (O.D.)
0 hr
5 hr
24 hr

Fig. 10a

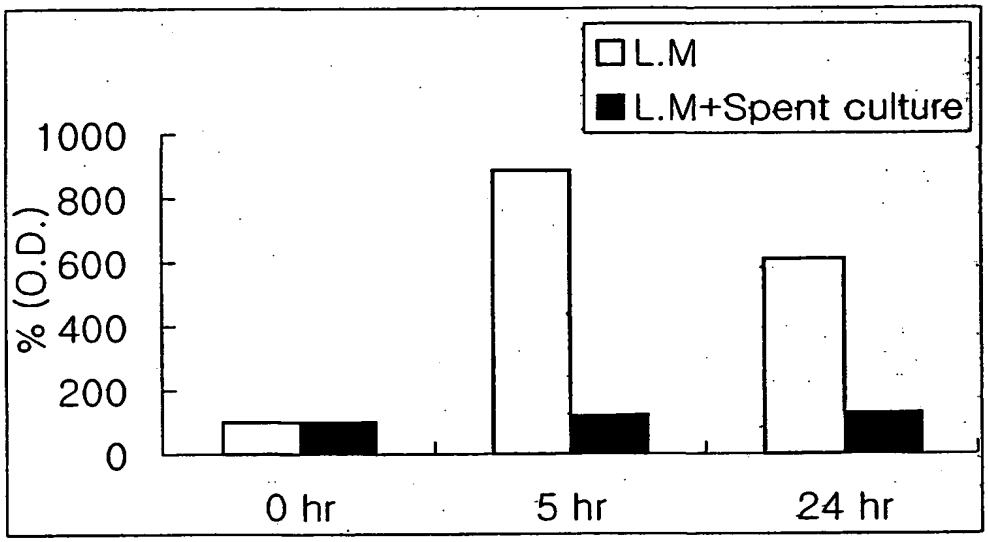


Fig. 10b

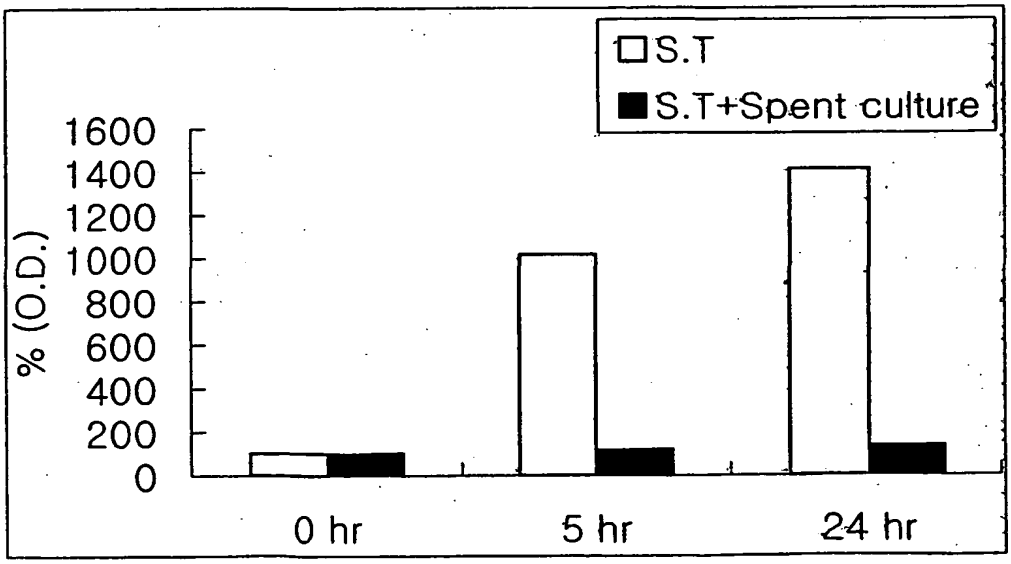


Fig. 10c

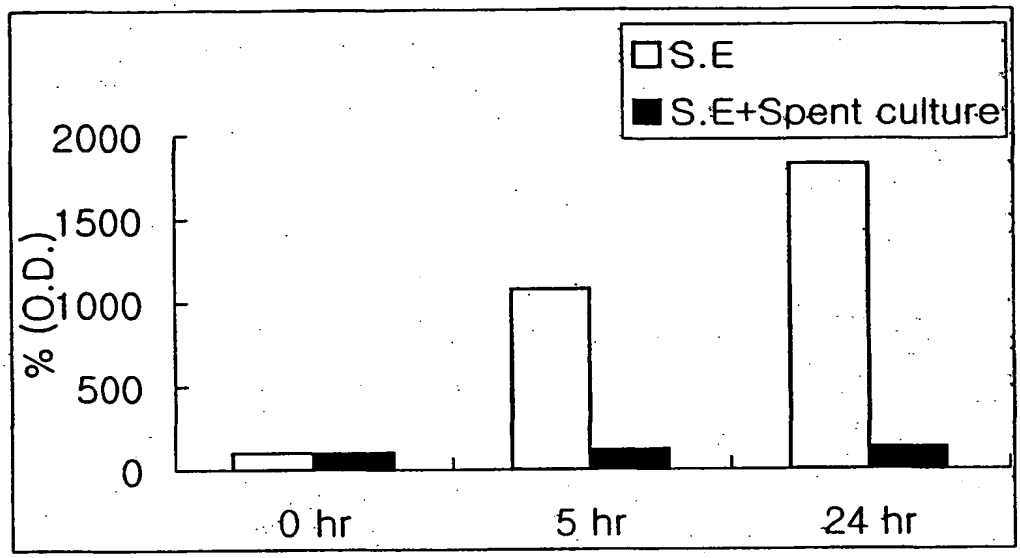


Fig. 10d

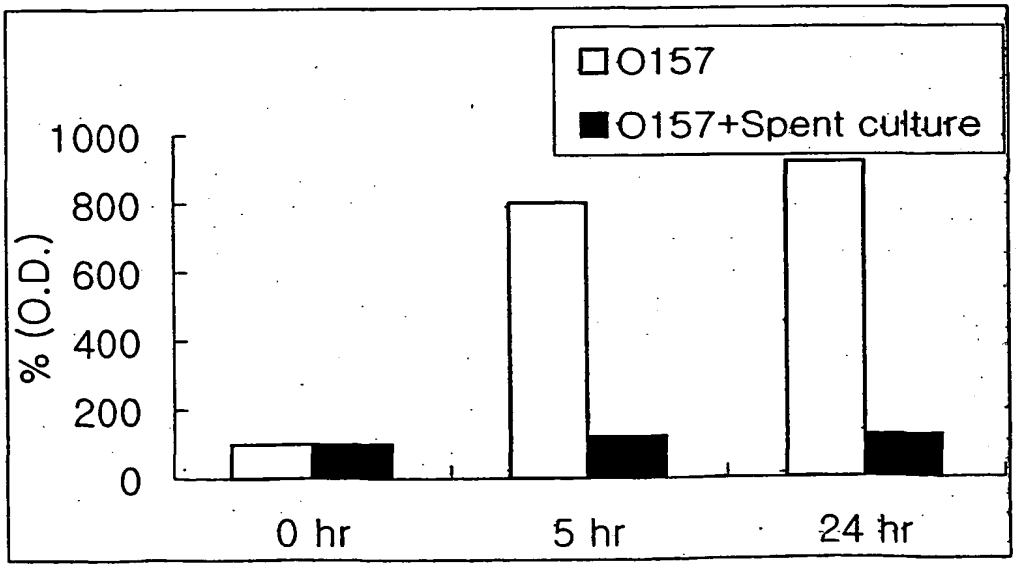


Fig. 10e

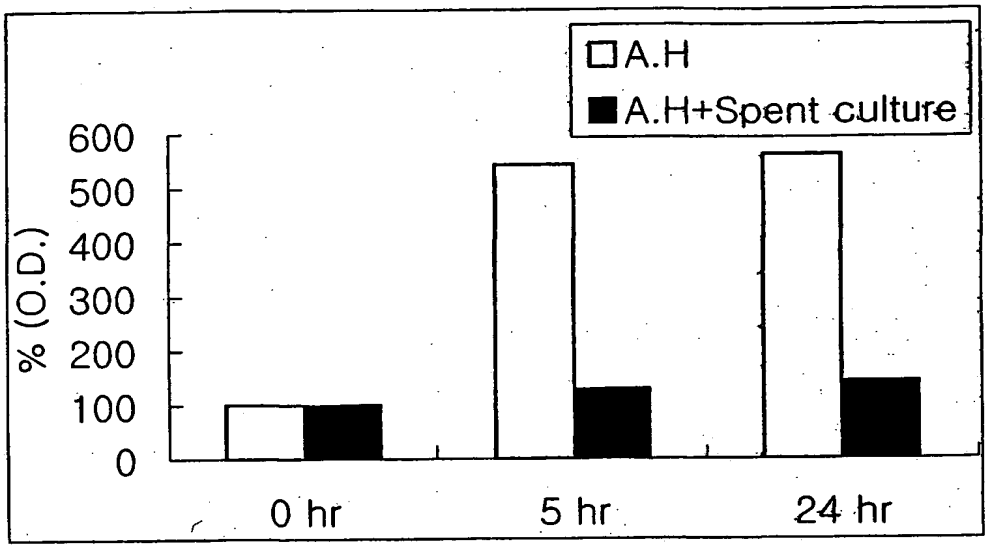


Fig. 11a

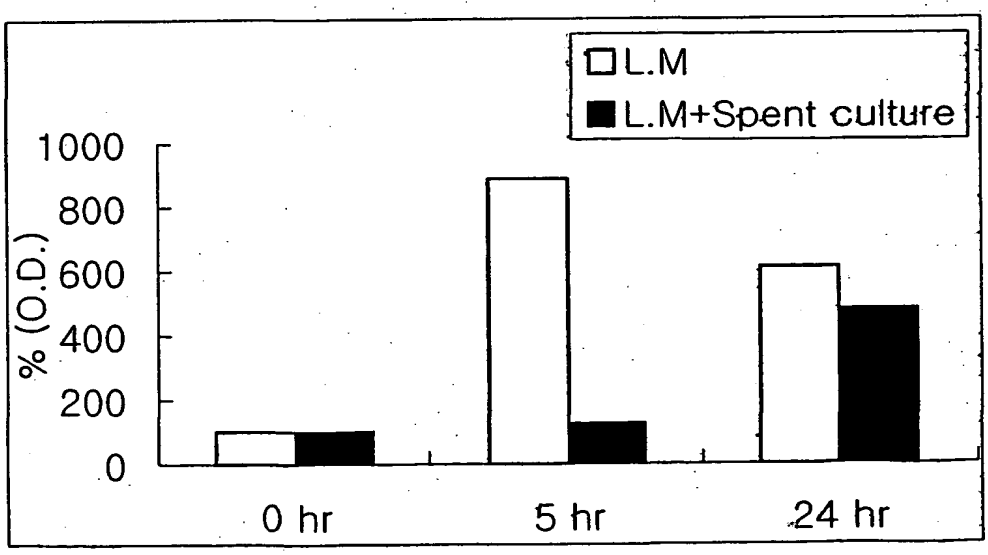


Fig. 11b

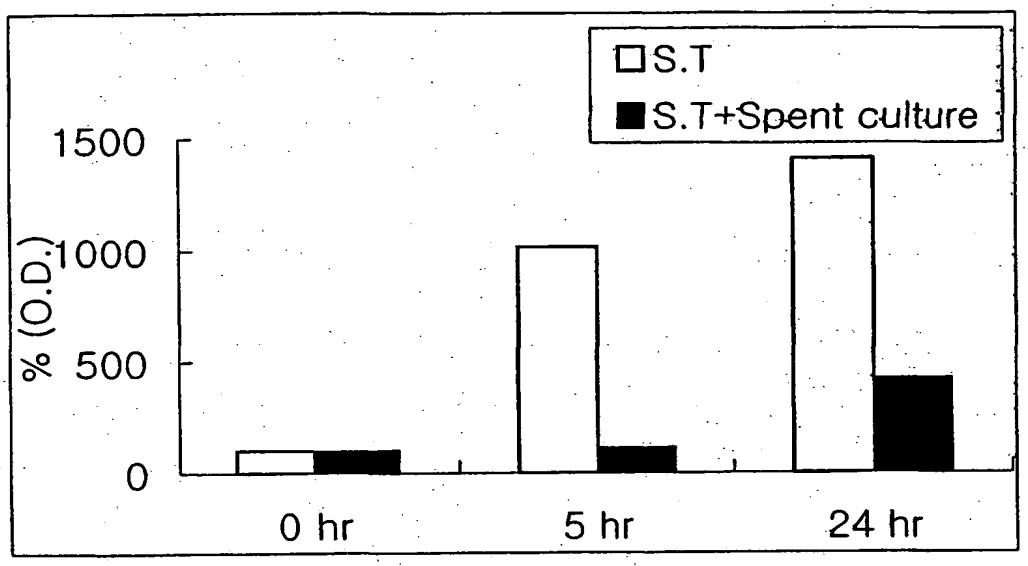


Fig. 11c

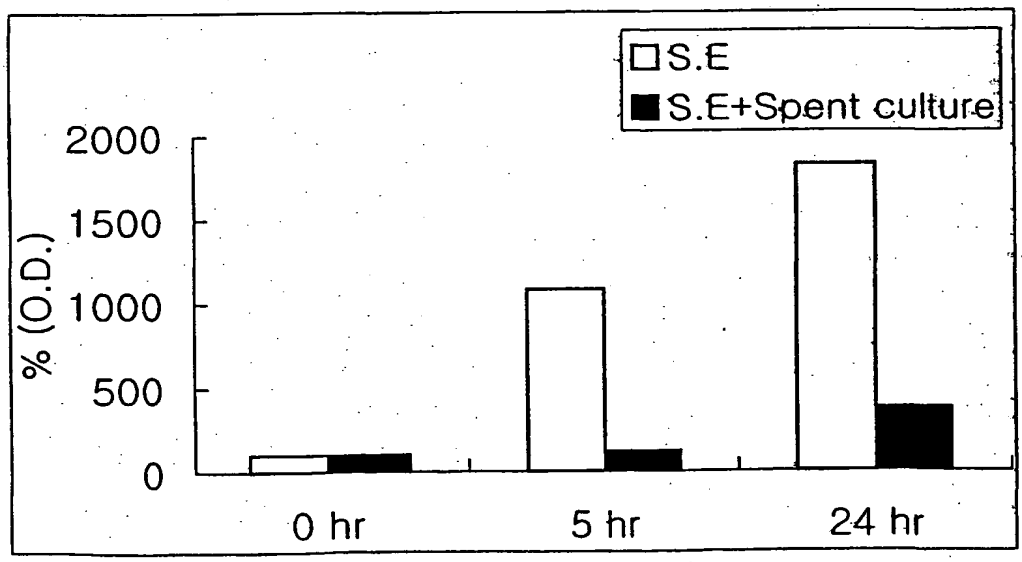


Fig. 11d

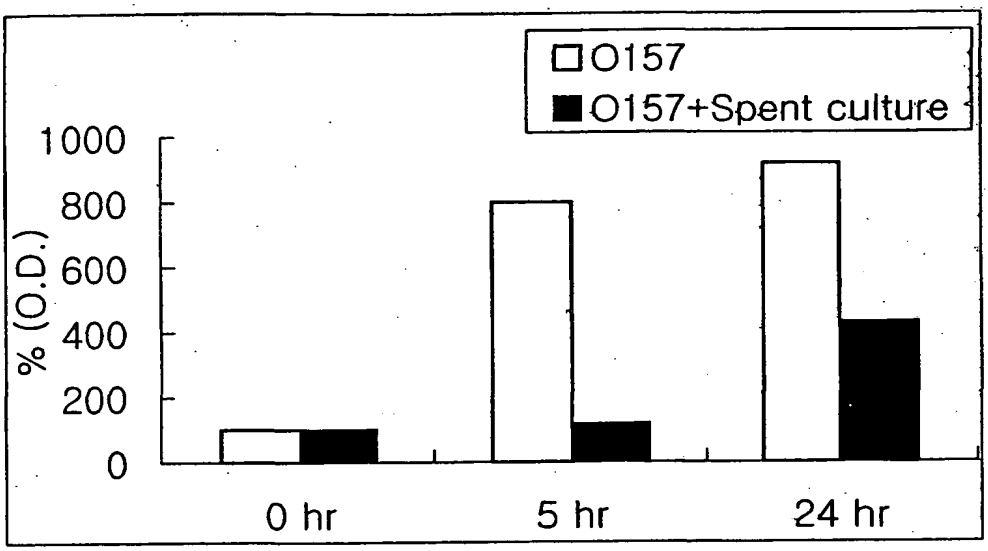


Fig. 11e

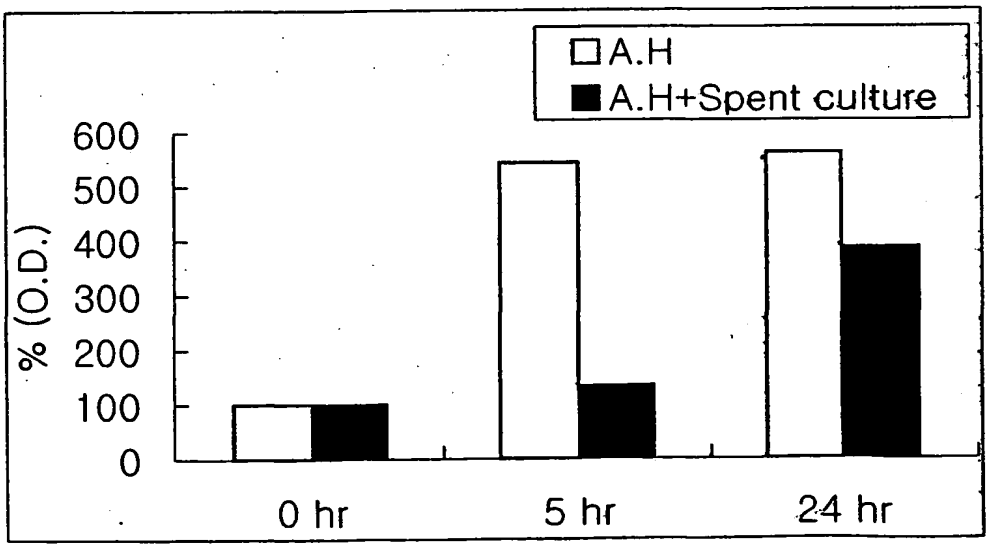


Fig. 12a

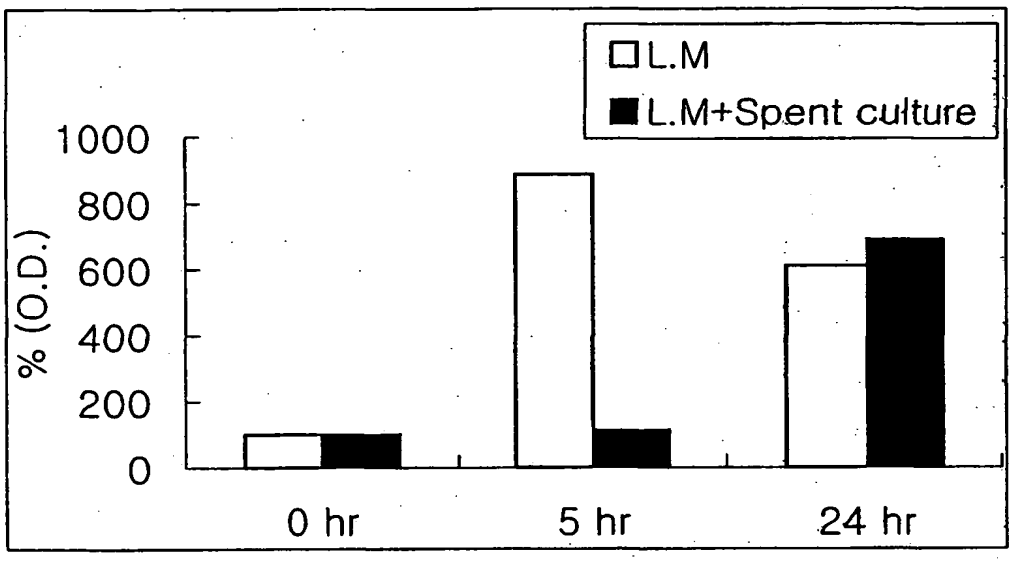


Fig. 12b

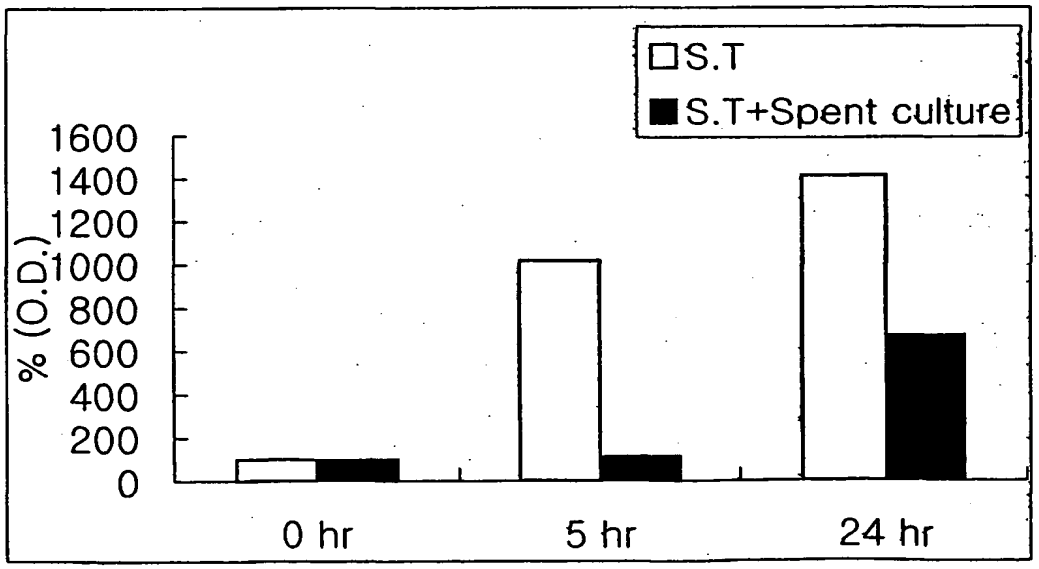


Fig. 12c

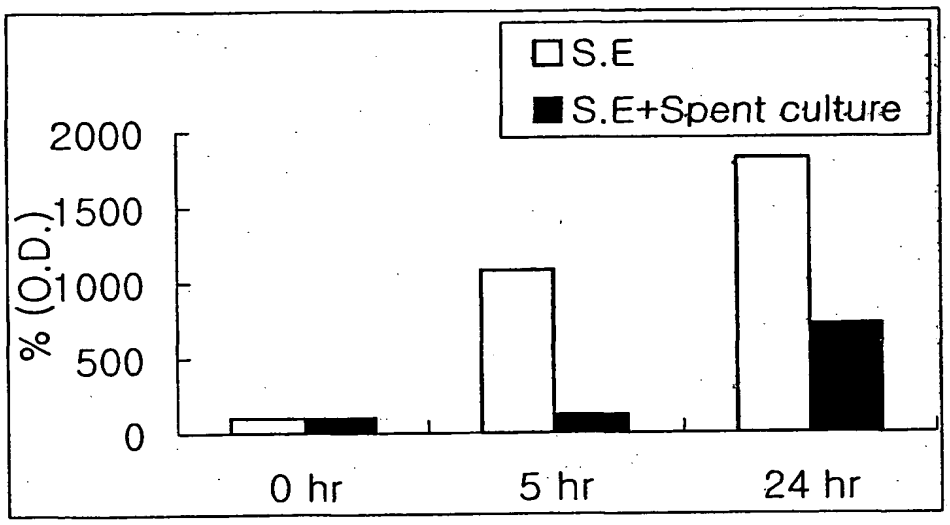


Fig. 12d

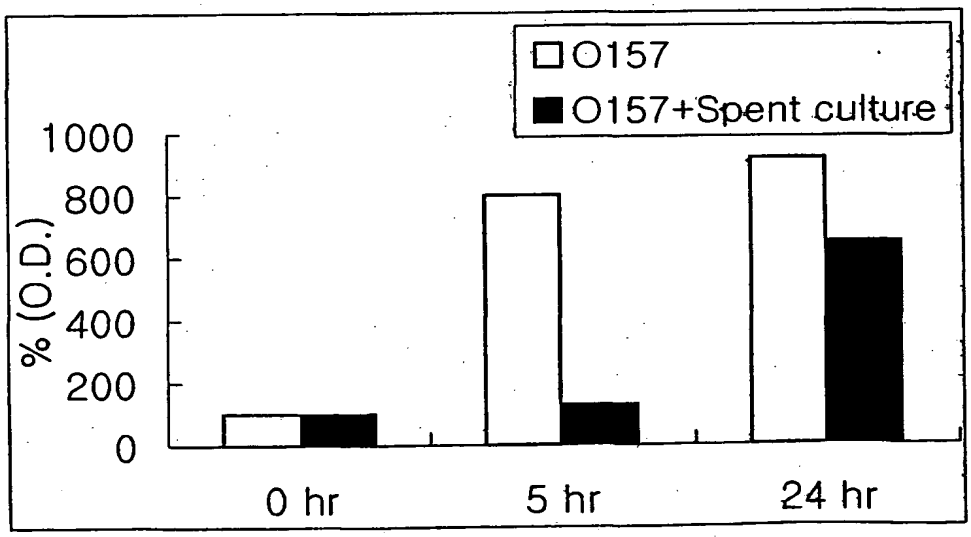


Fig. 12e

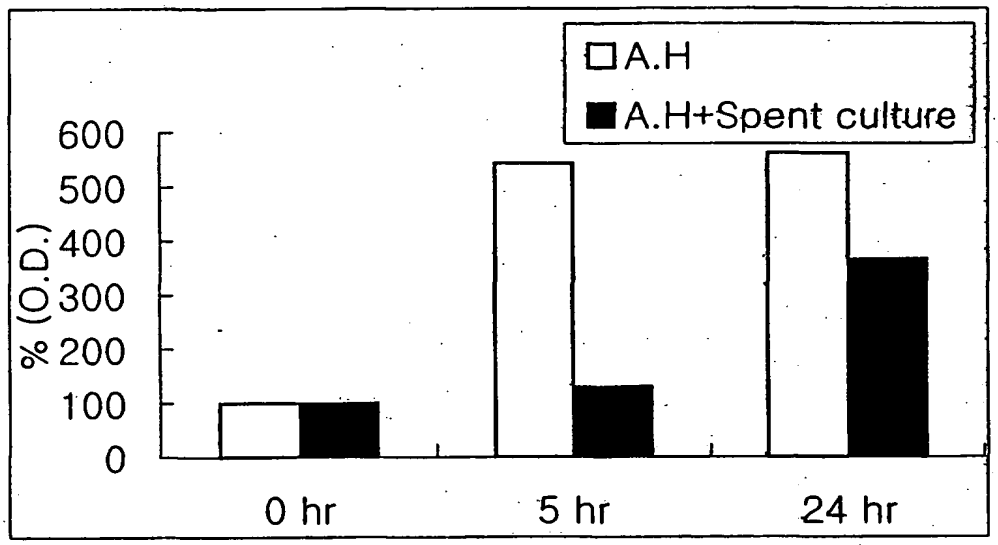


Fig. 13a

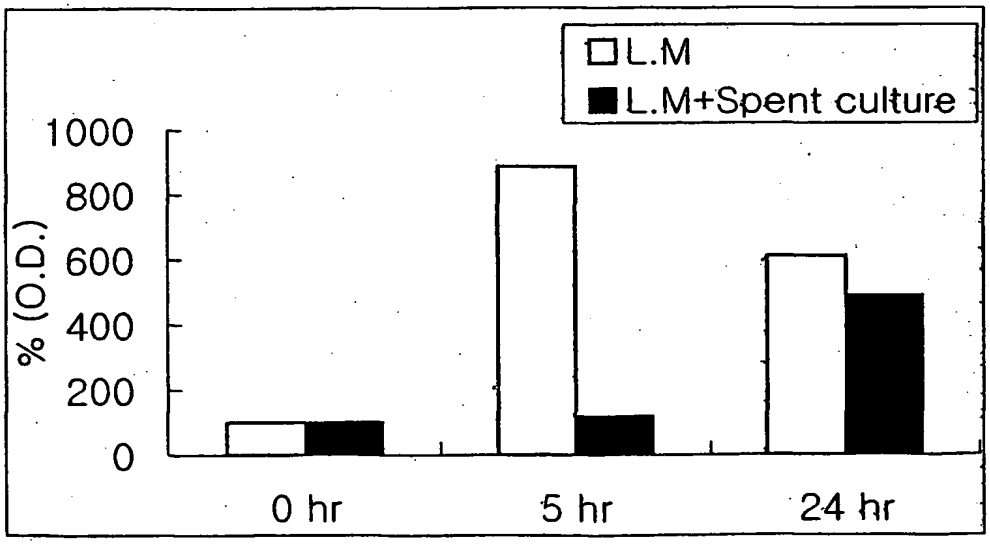


Fig. 13b

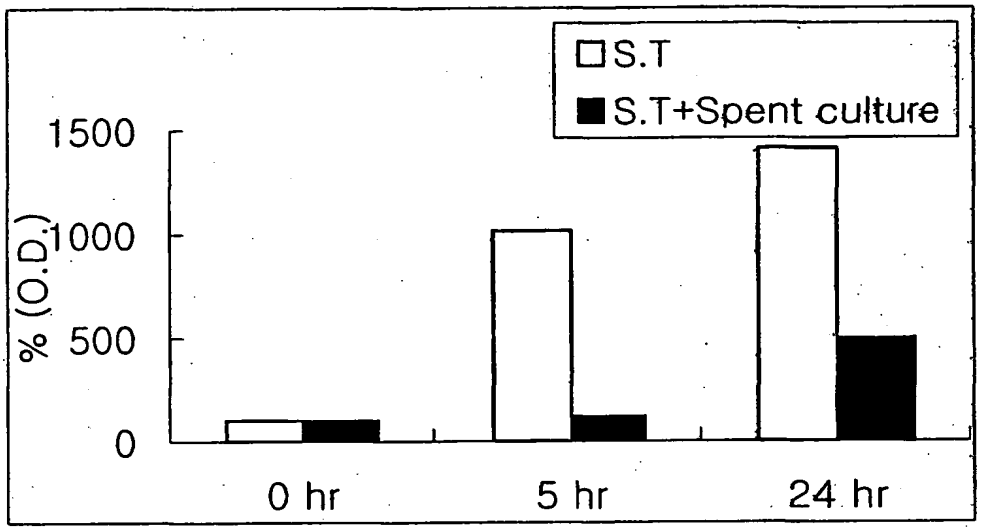


Fig. 13c

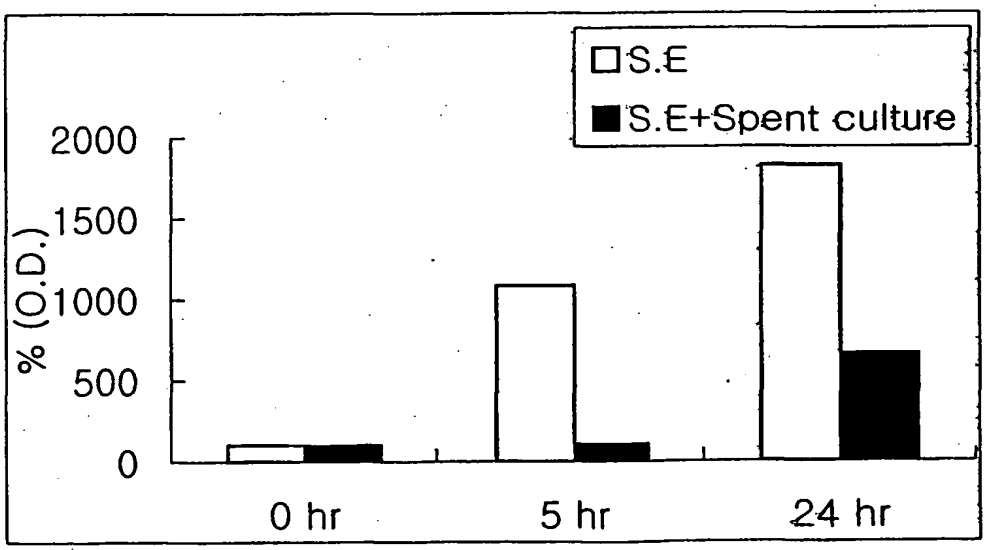


Fig. 13d

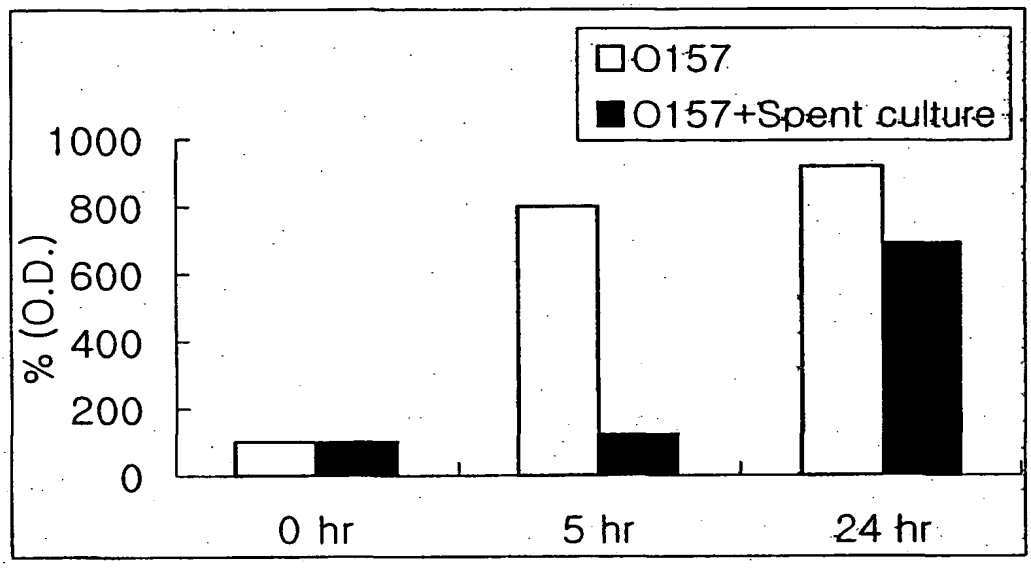


Fig. 13e

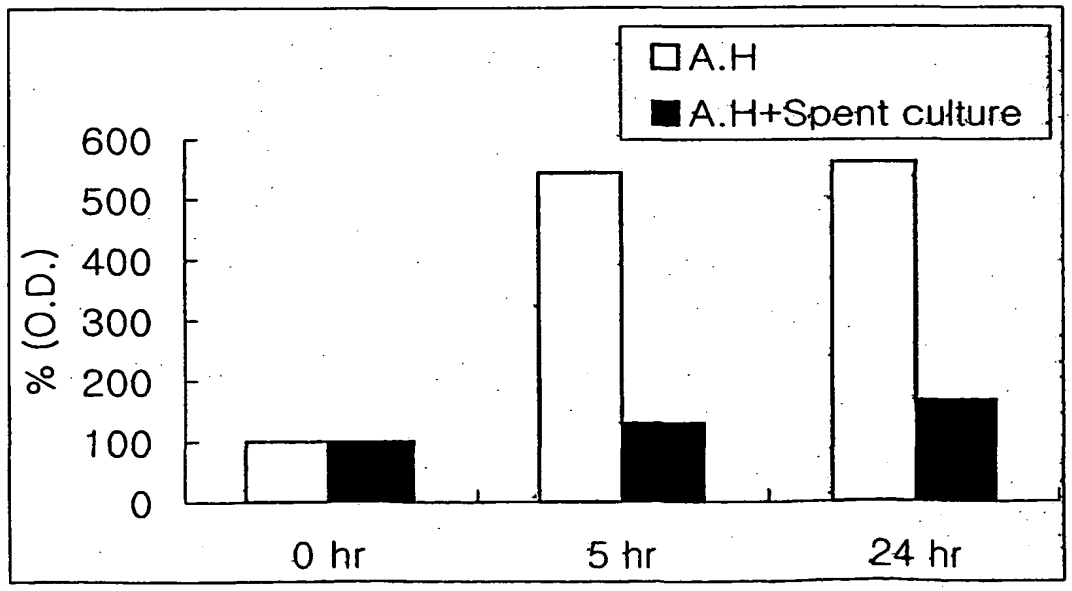


Fig. 14

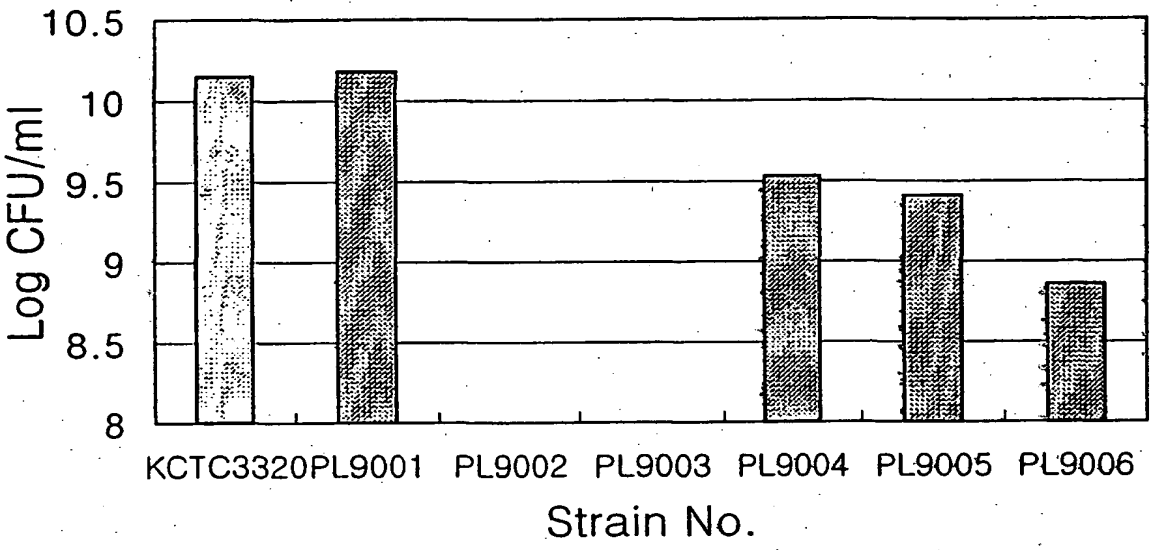


Fig. 15a

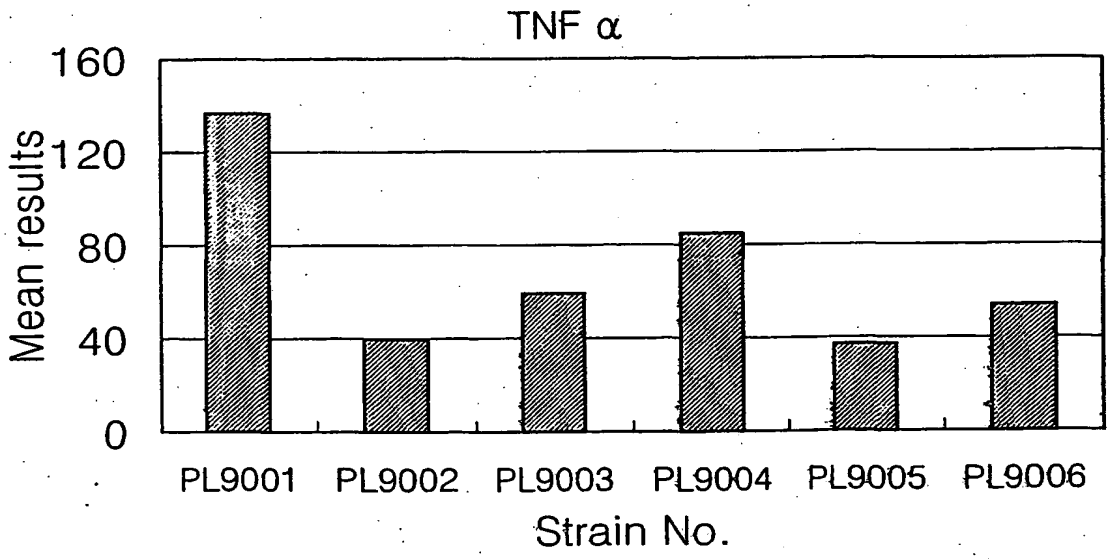


Fig. 15b

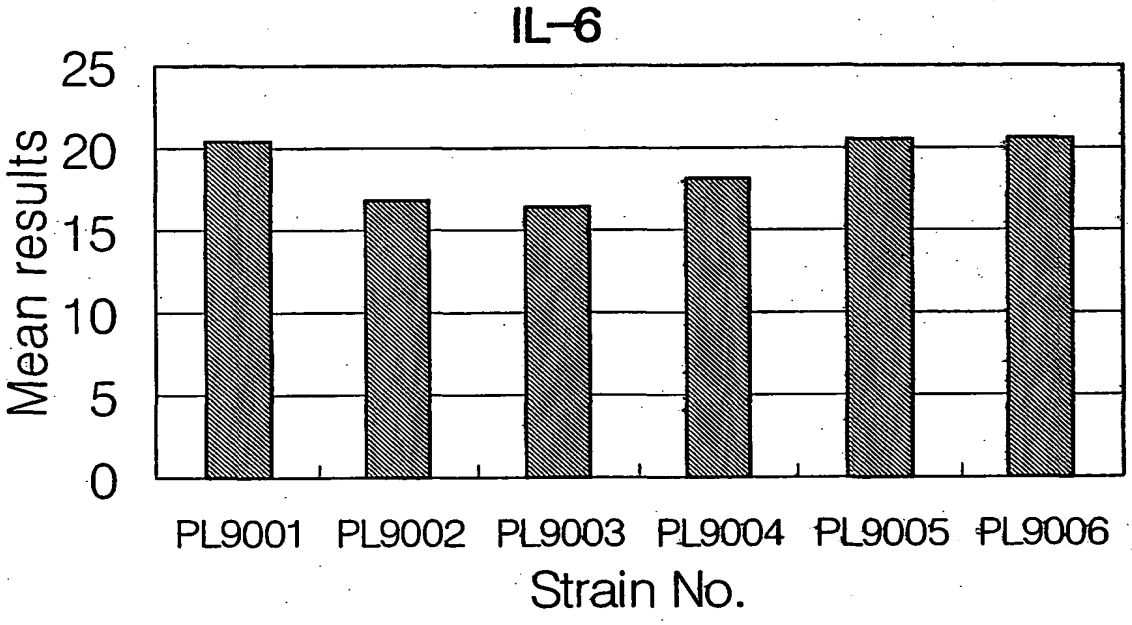

IL-6
25
20
15
10
5
0
Mean results
PL9001
PL9002
PL9003
PL9004
PL9005
PL9006
Strain No.

Fig. 16a

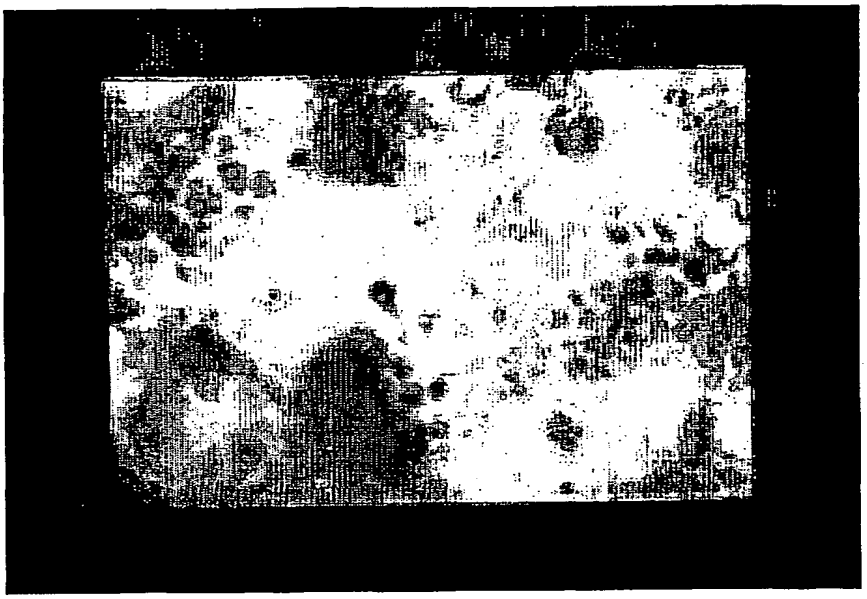


Fig. 16b

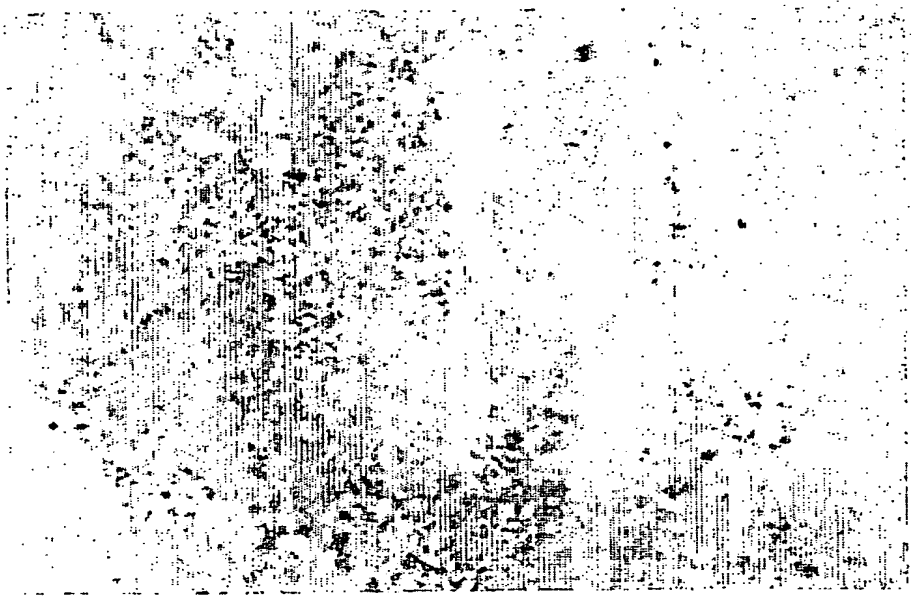


Fig. 16c

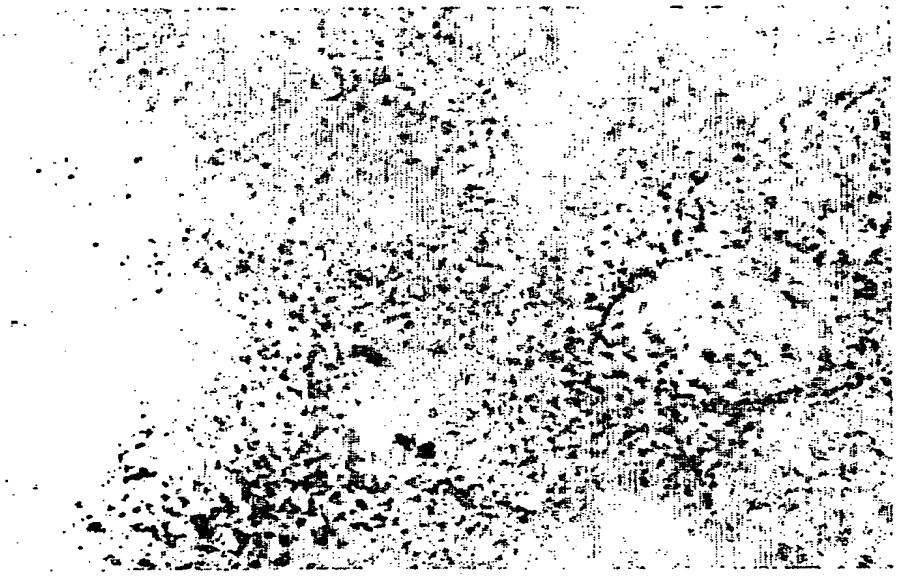


Fig. 16d

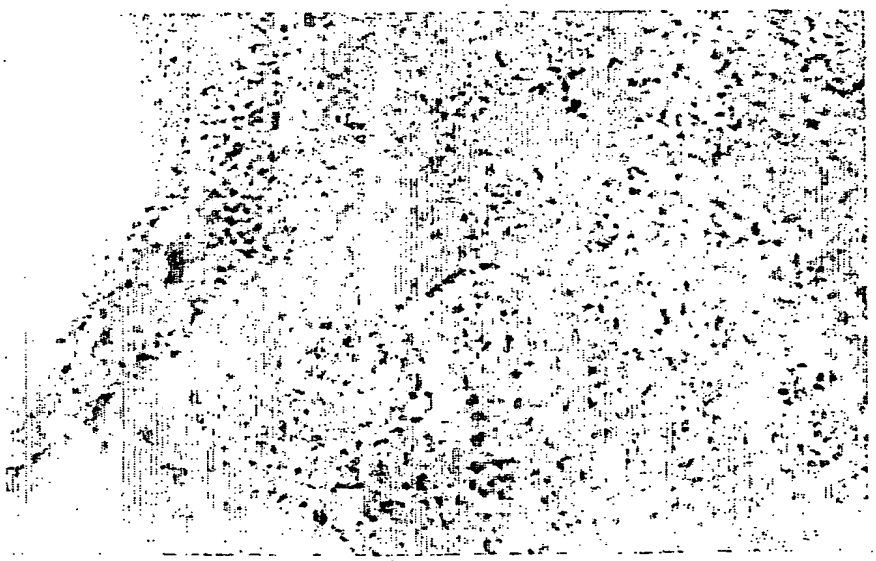


Fig. 16e

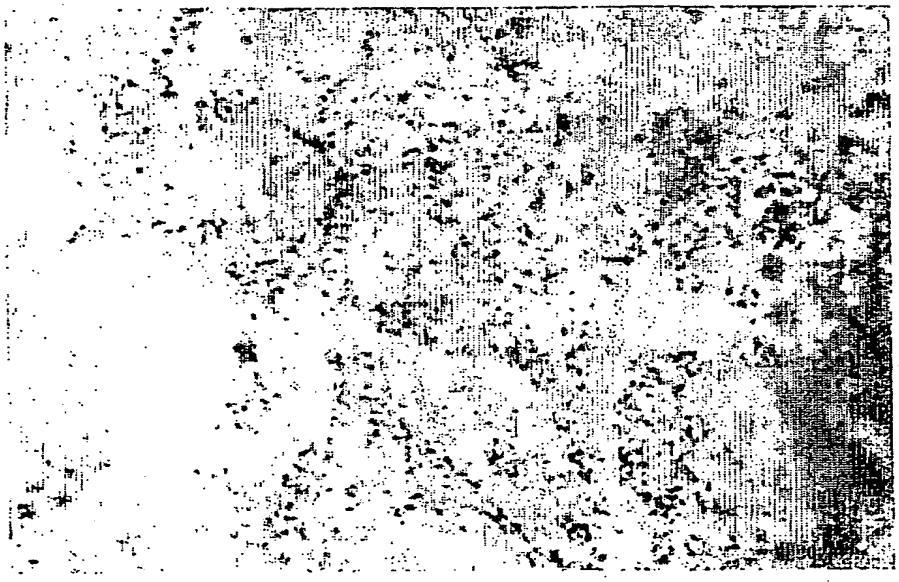
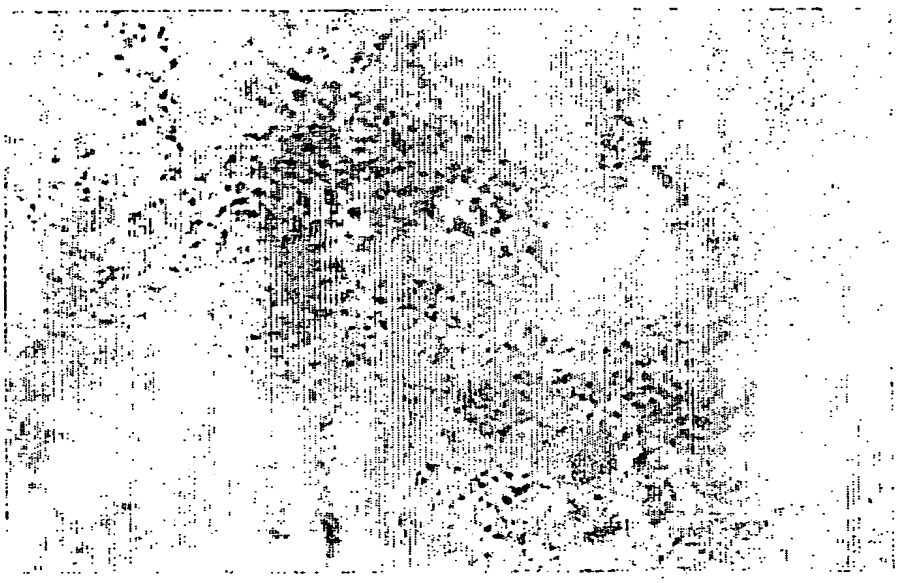


Fig. 16f



Sequence Listing

<110> PL BIO Co., Ltd

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gaaacctacc tcttagcagg ggataacatt tggaaacaga tgctaatacc gtataacaat 180

agcaaccgca tggttgctac ttaaaagatg gttctgctat cactaagaga tggtcccgcg 240

gtgcattagt tagttggtga ggtaatggct caccaagacg atgatgcata gccgagttga 300

gagactgatc ggccacaatg ggactgagac acggcccata ctcctacggg aggcagcagt 360

agggaatctt ccacaatggg cgaaagcctg atggagcaac gccgcgtgtg tgatgaaggg 420

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gaaggggata acacttggaa acaggtgcta ataccgtata acaatcgaaa ccgcatggtt 180

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acattgggac tgagacacgg cccaaactcc tacgggaggc agcagtaggg aatcttcggc 360

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cttgagtgca gaagaggaga gtggaattcc atgtgtagcg gtgaaatgcg tagatatatg 780

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gaacaacgct taaaagatgg cttctcgcta tcacttctgg atggacctgc ggtgcattag 240

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gggaatcttc cacaatgggc gcaagcctga tggagcaaca ccgcgtgagt gaagaagggt 420

ttcggctcgt aaagctctgt tgttaaa 447

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 01/02126

CLASSIFICATION OF SUBJECT MATTER

IPC[7]: A61K 35/74, C12N 1/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC[7]: A61K 35/74

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CAS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98/55131 A1 (GRAHN HAKANSSON, E. et al.) 10 December 1998 (10.12.98) *page 3, lines 6-14; page 4, lines 19-36; page 6, lines 17-30; page 7, lines 3-25; page 9, lines 8-12.*	1,3,4,6,8,9, 14-17,19,20
X	EP 1000625 A1 (LABORATOIRE DU LACTEOL DU DOCTEUR BOUCARD, INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE) 17 May 2000 (17.05.00) *abstract; page 1, lines 25-27; claims 1,3,4,6.*	1,3,4,6,8,9
X	EP 1038951 A1 (SOCIETE DES PRODUITS NESTLE S.A.) 27 September 2000 (27.09.00) *page 1, lines 6-10, 51-54.*	1,3,4,6,8,9, 14-16,19,20
X	JP 09 241173 A (WAKAMOTO PHARM CO LTD) 16 September 1997 (16.09.97) (abstract) WPI [online]. London, U.K.: Derwent Publications, Ltd. [retrieved on 13-02-2002]. DW 199750, AN: 1997-539731 [50] *abstract.*	1,3,4,6,8,9, 14-16,19,20

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Date of the actual completion of the international search	Date of mailing of the international search report
14 February 2002 (14.02.2002)	14 March 2002 (14.03.2002)
Name and mailing adress of the ISA/AT Austrian Patent Office Kohlmarkt 8-10; A-1014 Vienna Facsimile No. 1/53424/535	Authorized officer MOSSER Telephone No. 1/53424/437

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 01/02126

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 63 179829 A (ADVANCE KK) 23 July 1988 (23.07.88) (abstract) WPI [online]. London, U.K.: Derwent Publications, Ltd. [retrieved on 13-02-2002]. DW198835, AN: 1988-246702 [25] *abstract.*	10,12,13

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 01/02126

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EP A1 1000625	17-05-2000	FR A1 2785190	05-05-2000
		FR B1 2785190	26-01-2001
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		US BA 6340585	22-01-2002
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		AU B2 726217	02-11-2000
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		SE A 9702083	03-12-1998
		SE C2 511524	11-10-1999

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